# Studies on Simuliidae (Diptera), with particular reference to *Austrosimulium tillyardianum*

Volume 1

A thesis presented for the
degree of Doctor of Philosophy in Zoology
in the University of Canterbury,
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by

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'In a general way the habits of the Simuliidae are pretty well known and the group is so homogeneous that we can expect little that is novel even from a good observer in another part of the globe.'

Knab (1911) *Proceedings of the Entomological Society of Washington* 13:172

## Preface

The aim of my thesis is to present information on population changes in the common New Zealand simuliid *Austrosimulium (Austrosimulium) tillyardianum* Dumbleton. Larvae of this species live in riffle areas of open-country streams. Before this aim could be achieved, it was necessary to define the identity of the study animal and its relationships with other New Zealand Simuliidae, and gain an understanding of its life history.

Wainui Valley Stream (43° 49'S, 172° 54'E), Banks Peninsula was chosen as the study area to undertake these investigations on population changes (p.165). *A. tillyardianum* was the only simuliid present in the stream, larvae were readily available throughout the year, and also there were few other species present thus reducing the amount of possible interspecific interactions. The stream was sufficiently small in size to simplify sampling procedures. Counteracting these favourable aspects were the unfavourable factors of the Wainui Valley being a popular holiday area, and the presence of a Y.M.C.A. camp site by the stream. Because of these two factors, it was considered inadvisable to leave equipment at the stream to monitor continuously stream temperature and water level which were thought to influence population size.

The results of my investigations are contained in the series of papers which follow. The three main papers of the study are presented first, followed by four supporting papers on aspects not restricted to *A. tillyardianum*. Finally, three general interest papers and data of the instar determination and population sampling studies are included as appendices. Each paper is complete in itself, and has been written in the style and format of the journal in which it has been (or will be) published, or to which it will be submitted.

## Main papers

1. Taxonomy and life history stages of *A. tillyardianum*. In this paper all life history stages are described and figured. This is the first time that all stages of any simuliid species have been described in the same publication. Specimens of other simuliid species occurring in New Zealand were also collected during the study to help define the amount of variation

which could be expected within species and between species. After examination of this material I was certain that *A. tillyardianum* was the only simuliid present in the Wainui Valley Stream.

2. Instar determination. The nine larval instars of *A. tillyardianum* are shown to be biometrically distinguishable, and that larval instars separated on the basis of measurements correspond to the larval instar groupings separated on the basis of morphological characters. Nine larval instars is the highest recorded for any simuliid, and hence a detailed study on instar determination was required. Four methods of analyzing measurements are critically evaluated: frequency distributions, Brooks' rule (Dyar's rule), Student's t-test, and multiple discriminant function analysis. Previous instar determination studies are evaluated, and most are shown to be capable of being re-interpreted to give a higher number of larval instars.

3. Population changes. A 10 x 1 m experimental channel was constructed in a bend of the Wainui Valley Stream, and the bed was covered with small stones to be used as sampling units. Weekly sampling over a seven month period when stream temperatures were relatively uniform was undertaken. In this time nine cohorts were followed through their life histories. Although every larval instar was collected on each sampling occasion, the cohorts could be separated by changes in instar composition of samples between weeks as shown by the positions of "peaks" and "troughs". It was concluded that intraspecific competition for attachment sites and floods were the main factors influencing the size of *A. tillyardianum* populations.

## Supporting papers

4. Food of two New Zealand Trichoptera. *Hydrobiosis parumbripennis* and *Hydropsyche colonica* appeared to be the only potential invertebrate predators present in the Wainui Valley Stream that could affect *A. tillyardianum* numbers. However, gut analyses revealed that their predation on *A. tillyardianum* was unimportant.

5. Trichomycetes. A commensal fungus *Harpella melusinae* was found to be common in the digestive tracts of *A. tillyardianum* larvae, although it did not appear to affect the larvae. The fungus was also found in other simuliid species collected in New Zealand. This is the first Southern Hemisphere recording of *H. melusinae*, as is also the case for two other Trichomycetes found incidentally in the hindgut of *A. tillyardianum* larvae.

6. Wing and haltere venation of larvae. The main features of the larval and adult wing venation of the *Austrosimulium australense* group are shown to correspond, but two differences are of interest in terms of the phylogeny of the genus *Austrosimulium* and the proposed homology for a vein. The presence of a venation pattern in haltere buds of larvae is interpreted as further evidence for the view that halteres of Diptera are highly modified hind wings.

7. Dyar's rule predated by Brooks' rule. The rule credited to Dyar - that there is a geometric increase in the size of a sclerotized structure with each instar - is shown to have been formulated before Dyar by Brooks.

## Appendices

Appendix 1. The historical use of the common names "sandfly" and "black fly" for species of Simuliidae is outlined.

Appendix 2. Gynandromorph of *Austrosimulium australense*. This is the first report of a primary somatic hermaphroditic simuliid; that is, an individual possessing parts of the internal and external secondary sexual apparatus of both sexes.

Appendix 3. Obtaining literature through interlibrary loan. This summarizes my use of the University of Canterbury interloan system. Information on time for interloans to arrive is the first published from the point of view of a user. Compilation of the data for this paper was initially for use in a survey of New Zealand university library resources.

Appendices 4 and 5. Unpublished data. Original and derived data for the instar determination study are listed, followed by the data collected in the Wainui Valley Stream sampling programme.

ACKNOWLEDGMENTS

I wish to thank my supervisors Drs M.J. Winterbourn and V.M. Stout for their assistance and discussions during the course of this study, and also Professor E.C. Young (now at the University of Auckland) for his initial supervision. Other assistance received from individuals has been acknowledged in the different papers.

Specialized advice received from the Technical Staff and the provision of equipment for the study by them is gratefully acknowledged. I also thank colleagues who helped in various ways, especially G. Habib, G.S. Knight, F.H. Wood and Abdul Moeed.

Financial support for the study was provided by a New Zealand Postgraduate Scholarship.

Finally, I am grateful to my family and friends for their understanding and interest in this study over the years, and who helped to make it a worthwhile experience.

Contents

Volume 1

'How often an inquirer asks "What is this organism?" and, on being told "*Melania alba*", considers himself somehow wiser than before. Anthropologists know well the supposed power conferred by knowledge of a name.'

Johnson (1968) *Proceedings of the Linnean Society of New South Wales* 93:10

Paper 1

# Life history stages and taxonomy of *Austrosimulium (Austrosimulium) tillyardianum* (Diptera : Simuliidae)

Accepted for publication in: *New Zealand Journal of Zoology*
1(1), 1974

The male, female, pupa, ninth instar larva, and egg of *Austrosimulium (Austrosimulium) tillyardianum* Dumbleton, 1973 are redescribed, and larval instars one to eight are described and morphologically differentiated for the first time. The relationship of *A. (A.) tillyardianum* to other New Zealand species is briefly reviewed.

## INTRODUCTION

*Austrosimulium (Austrosimulium) tillyardianum* Dumbleton, 1973 was first described by Tonnoir (1925) under the name of *tillyardi*, and brief descriptions were given of the male, female, pupa, final instar larva and egg. Dumbleton (1973) amplified the original descriptions and noted that the name *tillyardi* was already preoccupied in *Austrosimulium* by an earlier use of the name by Tonnoir (1923a) (as *Simulium tillyardi*), and hence the new name *tillyardianum* was required (see p. 3).

The redescription of *tillyardianum* by Dumbleton was in two parts; the first part was given in a general description of the New Zealand species of *Austrosimulium*, whereas the second part described the diagnostic features of *tillyardianum* itself. Although many new features of *tillyardianum* were described, many characters of use for comparing *tillyardianum* with other world species were not mentioned. Further, the larval instars of *tillyardianum*, except the final, remained unrecognized and undescribed.

My work has shown that *tillyardianum* has nine larval instars in its life history. This was determined after detailed morphological examination of 342 larvae, and was verified by applying univariate and multivariate statistical techniques to ten measurements made on 289 of these larvae (in preparation). The discovery of nine instars for *tillyardianum* was surprising since it is the highest number recorded for any simuliid species, and is probably the highest instar number recorded for any nematoceran (Hennig, 1948). Most simuliids that have been studied are believed to have six (Terteryan, 1957; Harrod, 1964) or seven (Grenier & Feraud, 1960; Kaćanski, 1968; Jedlička, 1972) instars in their life histories, although two species have been reported to have only four instars (Yakuba, 1960), and one species has been shown to have eight (Smith, unpublished 1969).

It is not the purpose of this paper to present the methods and detailed results of the statistical analyses which showed *tillyardianum* had nine larval instars, but rather it is to provide a morphological description of all the life stages of *tillyardianum*. The reason for this description of *tillyardianum* is to complement previously published descriptions of the adults, pupa, 9th instar larva and egg, and to incorporate in the descriptions characters that could assist in the assessment of similarities and dissimilarities with other simuliid species. For the first time, larval instars 1 to 8 are described and morphologically differentiated. By fully

illustrating each of the life stages for the first time, many characteristic features of *Austrosimulium (Austrosimulium)* are given that may be used for a basis of comparison with other genera. The incomplete synonymy of Dumbleton (1973) is expanded.

## Previous taxonomic studies on *Austrosimulium*

The genus *Austrosimulium* was erected in 1925 by Tonnoir for the simuliids with 10-segmented antennae found in New Zealand and Australia. A South American species, *Paraustrosimulium anthracinum* (Bigot), has been assigned to *Austrosimulium* by several workers (Edwards, 1931; Wygodzinsky & Coscarón, 1962; Dumbleton, 1973), but this relationship has now been rejected (Crosskey, 1969; Wygodzinsky & Coscarón, 1973).

Since Tonnoir, taxonomic studies on *Austrosimulium* have been carried out in Australia by Mackerras & Mackerras (1948-55) and in New Zealand by Dumbleton (1963a, 1973). Additionally, diagnoses of *Austrosimulium sensu lato* have been given by Edwards (1931), Smart (1945), Mackerras & Mackerras (1949), Wygodzinsky & Coscarón (1962), Stone (1963), and Dumbleton (1963a, 1973), whereas Crosskey (1969) has enumerated the differences between *Austrosimulium* and the genera of Simuliini occurring in Africa.

*Austrosimulium* and *Gigantodax* were combined in the tribe Austrosimuliini by Smart (1945), but subsequently Crosskey (1969) has considered that *Austrosimulium* belongs in the tribe Simuliini and that *Gigantodax* should be assigned to the tribe Prosimuliini; this arrangement is accepted.

## Tonnoir's use of *"tillyardi"*

In 1923 Tonnoir described and illustrated the process of cocoon construction by a mature larva, and the hatching of an adult in a species he called *Simulium tillyardi*. In fact the species was *Austrosimulium australense* (Schiner) (Dumbleton, 1973). Since, under Article 16 of the "International Code of Zoological Nomenclature", Tonnoir provided a valid indication for *tillyardi*, the species must be considered as described and hence taking authorship. Thus the species *A. tillyardi* (Tonnoir, 1923), from the date of its publication, became a synonym of *australense* (Schiner, 1868).

In 1925, Tonnoir formally described a species under the name of *Austrosimulium tillyardi*, but this was not the *tillyardi* of the 1923 paper. Tonnoir (1925) stated that he had published a note on ". . . how one of the New Zealand species builds its cocoon" but the species was not mentioned by name. Presumably Tonnoir realized that he had misidentified his *tillyardi* of 1923, but probably did not realize that his use of *tillyardi* constituted a valid description, and therefore his *tillyardi* of 1925 should have been described under another name. Dumbleton (1973) briefly outlined the nomenclatural problems of *tillyardi*, and renamed *tillyardi* Tonnoir, 1925 as *tillyardianum*.

The misuse of *tillyardi* by Tonnoir has resulted in two different species being called *tillyardi* by subsequent workers (see synonymy for authors). Those authors describing cocoon construction and the hatching of the adult are using *tillyardi* to denote the species *australense* (Schiner), whereas the remaining authors are using *tillyardi* to mean the species *tillyardianum* Dumbleton. It is likely that this confusion will persist in the literature for some time to come.

## MATERIALS AND METHODS

All descriptions are based on specimens collected from the Wainui Valley Stream, Banks Peninsula, Canterbury (43° 49'S, 172° 54'E), although specimens of *tillyardianum* were examined from throughout its geographical range. The restriction to one locality for the description was for two reasons: firstly, *tillyardianum* was the only species present in the Wainui and thus no confusion with larval instars of other species was possible, and secondly, since many well-studied "species" have now been found to be complexes of sibling species, a description based on material from the Wainui Valley Stream study area only is desirable (Dunbar (1972), for example, has recognized 16 chromosomally distinct entities, exhibiting biological differences in many cases, in the widely distributed African "species" *Simulium (Edwardsellum) damnosum* Theobald).

Terminology in the descriptions is that outlined by Crosskey (1969) and, for the larval head capsule, that of Craig (1969); consequently, many terms used in Dumbleton's (1973) description differ from those used in this description.

Specimens used for the descriptions were those that had been preserved in 90% ethanol. All drawings were made with the aid of a camera lucida; whole mounts of specimens were used for low magnification drawings, but specimens used for drawings made at higher magnifications were first cleared with 10% KOH before the required structures were mounted in glycerine.

## DESCRIPTIONS

### *AUSTROSIMULIUM (AUSTROSIMULIUM) TILLYARDIANUM* DUMBLETON,1973

*Austrosimulium (Austrosimulium) tillyardianum* Dumbleton,1973, *New Zealand Journal of Science* 15(4):513. Replacement name for *tillyardi* Tonnoir, 1925, *Bulletin of Entomological Research* 15(3):253, junior secondary homonym of *tillyardi* Tonnoir,1923a, *Annales de Biologie Lacustre* 11 (3-4):165 [= *australense* Schiner,1868, "Reise der Österreichischen Fregatte 'Novara' um die Erde" Zoologischer Theil. 2:15].

*Austrosimulium tillyardi* Tonnoir,1925, *Bulletin of Entomological Research* 15(3):253, (original description);---Pulikowsky 1929, *Zeitschrift für Morphologie und Ökologie der Tiere* 13:656-9;---Rubzov 1940, *Fauna SSSR* 6(6):117;---Smart 1945, *Transactions of the Royal Entomological Society of London* 95(8):499;---Mackerras & Mackerras 1949, *Proceedings of the Linnean Society of New South Wales* 73(5-6):404;---Miller 1950, *New Zealand Department of Scientific and Industrial Research Bulletin* 100:61;---Hennig 1950, "Die Larvenformen der Dipteren", Akademie-Verlag, Berlin, 2:384;---Wisely 1952, (Unpublished M.Sc. thesis, lodged in library of University of Canterbury, New Zealand) p.51;---Wisely 1962, *Transactions of the Royal Society of New Zealand, Zoology* 2(25):213;---Dumbleton 1963a, *New Zealand Journal of Science* 6(3):334;---Dumbleton 1963b, *New Zealand Entomologist* 3(2):35;---Wise 1965, *Pacific Insects* 7(2):208;---Craig 1966, (Unpublished Ph.D. thesis, lodged in library of University of Canterbury, New Zealand) chapter 3:23;---Dumbleton 1969, *In* Knox, G.A. (Ed.) "The Natural History of Canterbury", A.H. & A.W. Reed, Wellington, p.485;---Stout 1969, *In* Knox, G.A. (Ed.) "The Natural History of Canterbury", A.H. & A.W. Reed, Wellington, p.491; ---Dumbleton 1970, *New Zealand Entomologist* 4(3):20.

*Simulium (Austrosimulium) tillyardi*;---Grenier 1949, *Physiologia Comparata et Oecologia* 1:201(Fig.23), (name *tillyardi* not used, but figure corresponds to figure given by Tonnoir 1925:226(Fig.10F) for *tillyardi*).

Not *tillyardianum*, species referred to is *Austrosimulium (Austrosimulium) australense* (Schiner).

*Simulium tillyardi* Tonnoir,1923a, *Annales de Biologie Lacustre* 11(3-4):165; ---Tonnoir 1923b, *Bulletin de la Société Entomologique de Belgique* 5:85;---Puri 1925, *Parasitology* 17(3):301;---Wu 1931, *Papers from the Michigan Academy of Science, Arts and Letters* 13:552;---Bequaert 1934, *In* Strong, R.P.; Sandground, J.H.; Bequaert, J.C.; Ochoa, M.M. "Onchocerciasis with particular reference to the Central American form of the disease", Harvard University Press, Cambridge, p.189;---Smart 1934, *Proceedings of the Royal Physical Society of Edinburgh* 22(4):236;---Ussova 1955, *Doklady Akademii nauk SSSR* 105(4):846;---Burton 1966, *Annals of Tropical Medicine and Parasitology* 60(1):48.

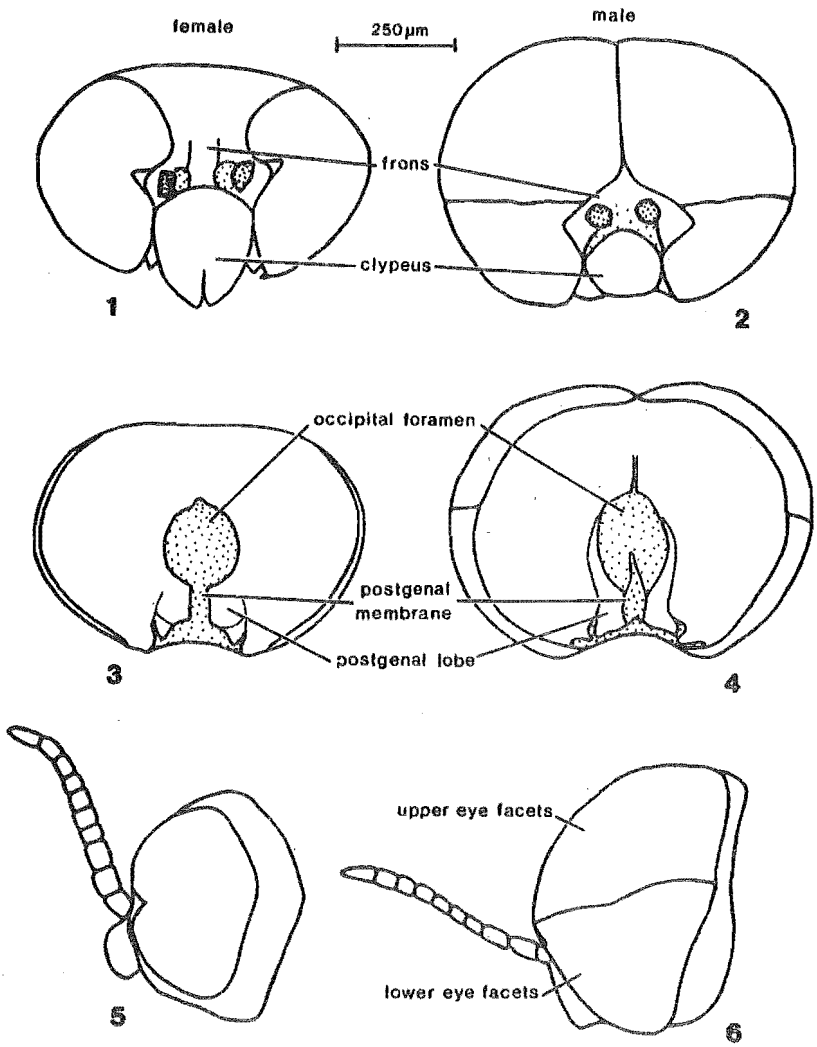
*Austrosimulium tillyardi*;---Mackerras & Mackerras 1948, *Australian Journal of Scientific Research (B)* 1(2):246;---Peterson 1956, *Canadian Entomologist* 88(8):500.

Medium sized species, approximate measurements:

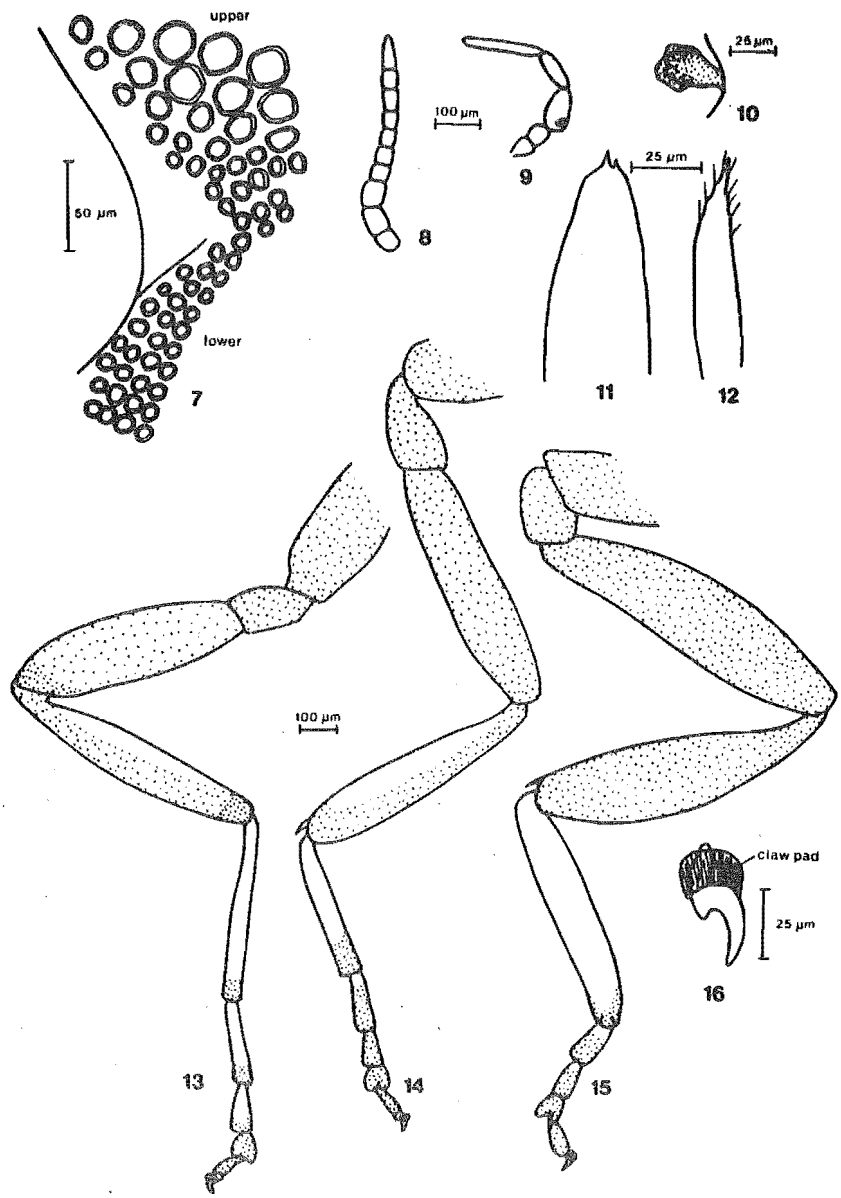
Male: body length 2.5-3.0 mm, wing length 2.2-2.5 mm.
Female: body length 2.6-3.1 mm, wing length 2.8-3.2 mm.
Pupa: body length 3.0-3.5 mm.
9th instar larva: body length 4.6-6.0 mm.
Egg: length 0.20-0.24 mm, greatest breadth 0.12-0.15 mm.

MALE: General body colour brownish-black; pinned specimens with overlying silvery pruinose appearance.

Head: Normal (Figs 2, 4, 6), wider than thorax. Head holoptic (Figs 2, 6), enlarged upper eye facets occupying 2/3 area of head, areas of lower eye facets and clypeus correspondingly reduced. About 20 vertical columns and 28 horizontal rows of upper eye facets; diameter of upper eye facets 2.0-2.5 times that of lower facets (Fig. 7). Clypeus dark brown, sparsely haired. Antenna 10-segmented (Fig. 8), pale yellowish-brown, covered with fine hair; scape and pedicel slightly enlarged, bearing hairs, these about 1/2 length of pedicel; segment 3 slightly smaller than pedicel; segments 4-9 small; segment 10 elongated cone. Maxillary palp (Fig. 9) 5-segmented, pale yellowish-brown, but segment 3 darker brown with longer hairs; segment 5 about 1.5 times as long as segment 3; Lauterborn's organ (Fig. 10) small and dark. Proboscis light brown; short, twice as long as wide. Mouthparts




**FIGS 1-6**--*Austrosimulium tillyardianum*, adult head form. (1) Female, facial aspect, antennae omitted. (2) Male, facial aspect, antennae omitted. (3) Female, posterior aspect. (4) Male, posterior aspect. (5) Female, profile. (6) Male, profile.




FIGS 7-16--*Austrosimulium tillyardianum*, male. (7) Fronto-ocular triangle and eye facets. (8) Antenna. (9) Maxillary palp. (10) Lauterborn's organ (sensory vesicle) of 3rd segment of maxillary palp. (11) Tip of mandible. (12) Tip of maxilla. (13) Fore leg. Stippling indicates regions of darker coloration. (14) Mid leg. (15) Hind leg. Calcipala and pedisulcus present. (16) Claw of hind leg.

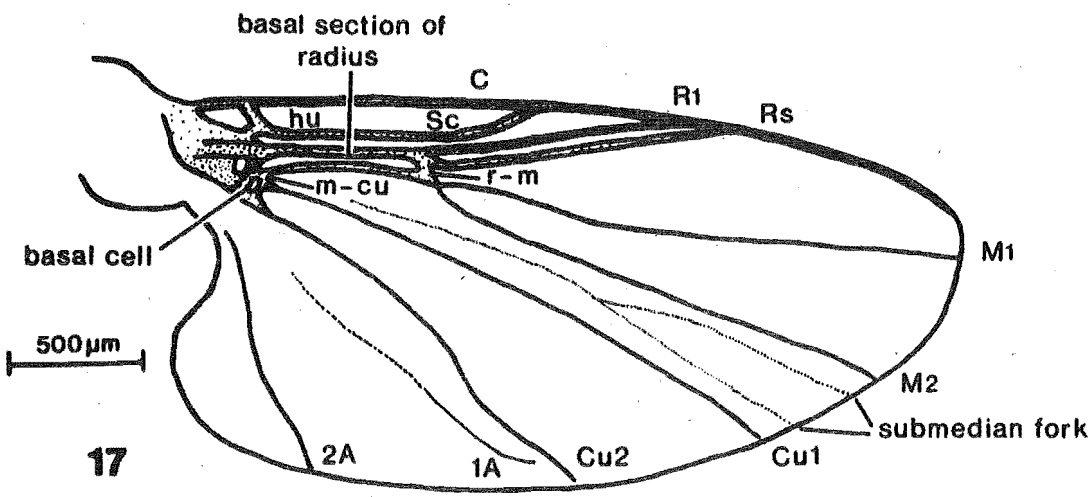
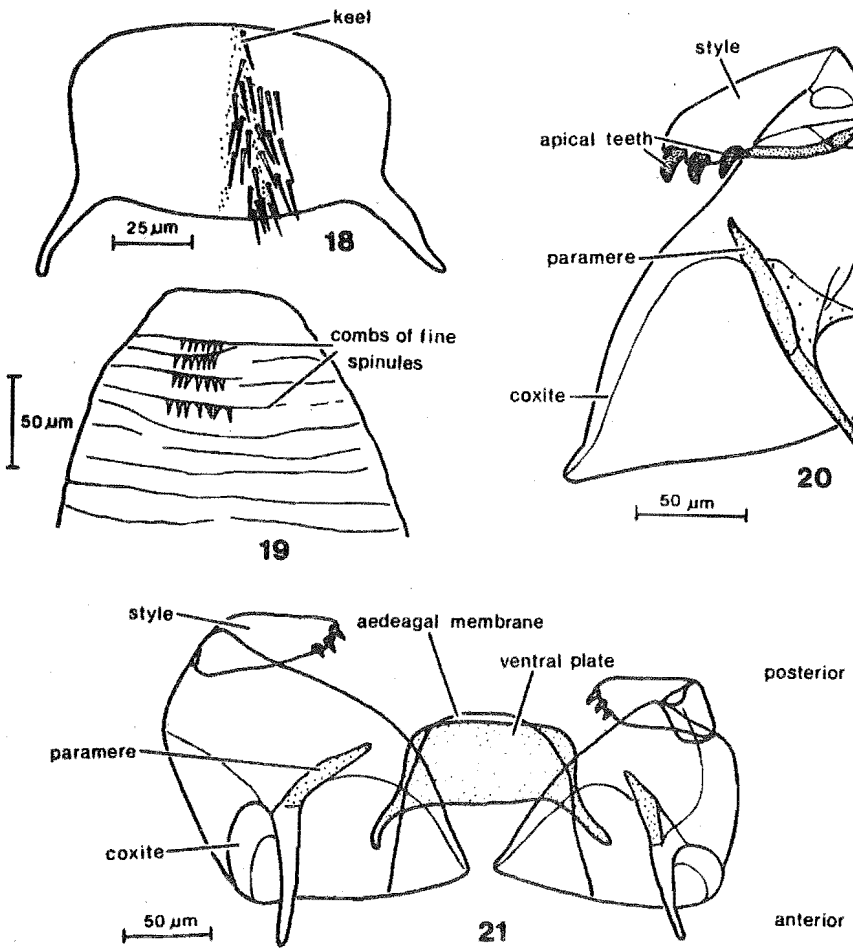



FIG. 17--*Austrosimulium tillyardianum* wing venation, female.




**FIGS 18-21--***Austrosimulium tillyardianum*, male. Genitalia in ventral view. (18) Ventral plate, with median keel. (19) Aedeagal membrane with combs of fine spinules. (20) Coxite, style and paramere. (21) Relationship between the structures comprising the male genitalia. The coxites and styles have been spread slightly.

poorly developed; mandible (Fig. 11) with 2 spine-like projections apically; maxilla (Fig. 12) with fine projections apically and subapically. Posterior surface of head (Fig. 4) with postgenal lobes separated by postgenal membrane; occipital foramen ovoid.

Thorax: Scutum without pattern, unicolorous dark reddish-brown; covered with evenly distributed brassy yellow (in pinned specimens) decumbent hair. Prescutellar depression with longer dark hairs. Scutellum with long black hair, more numerous laterally. Postnotum bare. Pleural membrane bare. Katepisternum bare. Mesepisternal sulcus deep, well defined. Legs (Figs 13-15): light yellowish-brown, with darker brown femoral, tibial and tarsal bases; hairs yellowish, short. Fore, mid and hind tarsus slender, parallel-sided; hind tarsus widest. Fore basitarsus about 8 times as long as its greatest breadth. Hind leg with pedisulcus and well developed calcipala; hind basitarsus about 5.0-5.5 times as long as its greatest breadth. Claw (Fig. 16) partially covered by a finely striate sclerotized pad extending distally from tarsus 5. Wings (Fig. 17): fore veins well developed, dark brown. Costal vein with well developed spinules as well as hairs (Fig. 35); spinules present from a point halfway between *hm* and *Sc* apex, to *C* apex; $R_1$ haired but without spinules. *Rs* not forked; $R_1$ and *Rs* meeting *C* about 2/3 from distal end of wing. *M* with short stem arising from *r-m*. $M_1$, $M_2$, $Cu_1$, $Cu_2$, *2A*, well defined; submedian fork and *1A* ill defined. $Cu_2$ with double curvature; basal section of radius haired; basal cell present; membrane evenly covered with normal microtrichia. Halteres brownish-black.

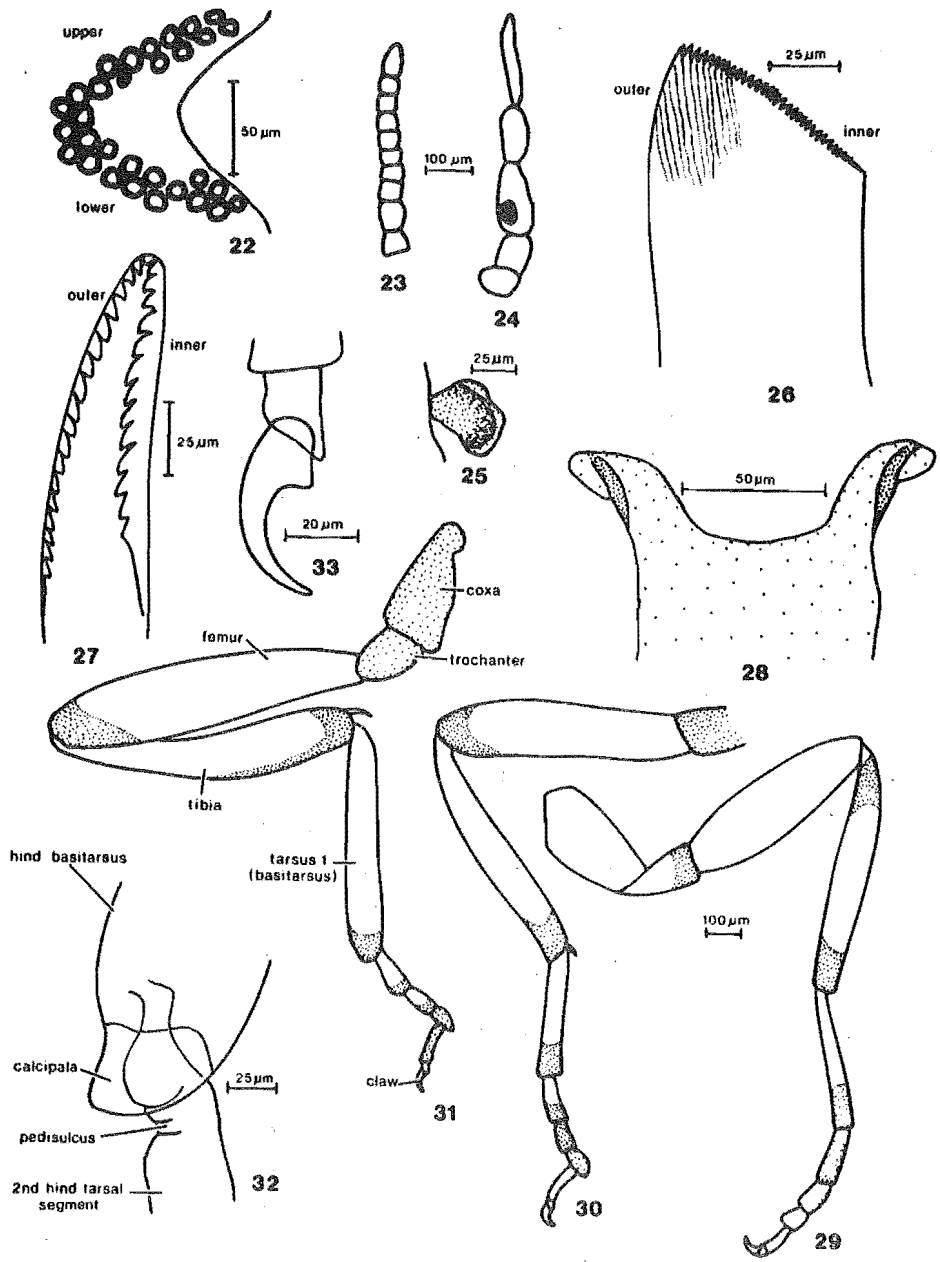
Abdomen: Tergites and sternites blackish-brown, lateral areas dull black. Tergites occupying whole width of dorsum; sternites 1, 3-9 present, 2 absent; sternite 1 small, semicircular; sternites 3-8 subquadrate, increasing in size from 3 to 8; sternite 9 small, semicircular, at base of coxites. Abdominal hairs present in pleural region, longest in mid-abdominal region. Genitalia (Fig. 21) of normal *Austrosimulium* type. Styles (Fig. 20) tapering and slightly truncate at tips, shorter than coxites, with 2 or 3 apical teeth; coxite not produced beyond base of style; ventral plate (Fig. 18) broad with median keel, all but posterior side haired, anterior arms short and stout; median sclerite not visible; parameres (Fig. 20) short, without hooks or spines; aedeagal membrane (Fig. 19) with forward-directed combs of fine spinules.

FEMALE: General body colour darker than male; pinned specimens with overlying silvery pruinose appearance, more pronounced than male.

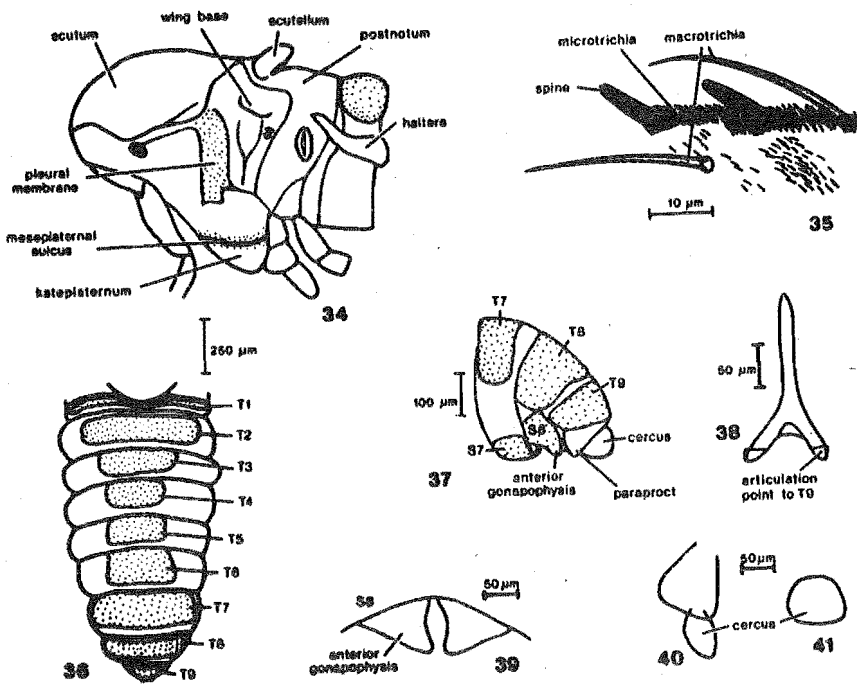
Head: Normal (Figs 1, 3, 5) slightly narrower than thorax. Head dichoptic (Figs 1, 3), eye facets uniformly small (Fig. 22). Frons with short hairs; narrowest just above fronto-ocular triangles, being about 1/4 of head width (Fig. 1); with median longitudinal depression. Clypeus dark brown (Fig. 1) with sparse hairing. Antenna 10-segmented, pale yellowish-brown, covered with fine hair; proportions of segments similar to male. Maxillary palp (Fig. 24) 5-segmented, similar to male. Lauterborn's organ (Fig. 25) small and dark; wider than male. Proboscis light brown, with dark brown sclerotized supports; short and stout, twice as long as wide. Mouthparts well developed, capable of biting man; mandible (Fig. 26) with about 35 inner teeth, increasing in size towards apex, no outer teeth; maxilla (Fig. 27) with about 20 outer and 15 inner inwardly directed teeth; posterior border of cibarium (Fig. 28) without spines or sculpturing in median area; well sclerotized lateral arms. Posterior surface of head (Fig. 3) with postgenal lobes separated by postgenal membrane; occipital foramen ovoid.

Thorax (Fig. 34): Scutum unicolorous semi-shiny dark reddish-brown; sparsely covered with decumbent hairs; in pinned specimens, a median and 2 lateral longitudinal dark lines visible between overlying silvery pruinosity. Other features as in male. Legs (Figs 29-31): yellowish-brown, with darker brown bases to femoral, tibial and tarsal segments; hairs short, yellowish. Fore, mid and hind tarsus slender, sub-parallel sided; hind tarsus widest. Fore basitarsus about 8 times as long as its greatest breadth. Hind leg with pedisulcus and well developed semicircular calcipala (Fig. 32), calcipala with a comb of small teeth as in male; hind basitarsus about 6.5-7.0 times as long as its greatest breadth. Claw (Fig. 33) without basal tooth. Wings (Figs 17, 35): as in male. Halteres light yellowish-brown.

Abdomen: Tergites and sternites blackish-brown, lateral areas dull greyish-black. Tergites (Fig. 36) of variable shape and size; tergites 2 and 7 largest, tergite 10 smallest and only visible caudally. Sternite 1 present, small; sternites 2-6 absent; sternites 7 and 8 present (Fig. 37); sternite 8 crescentic, convex posteriorly, lateral lobes acute and located between tergites 8 and 9. Tufts of abdominal hair in pleural areas, longest distally. Genitalia (Figs 38-41) of normal *Austrosimulium* type. Spermatheca ovoid, without surface pattern, and with internal hairs 5-7 µm long usually arranged in short rows; spermathecal duct not sclerotized. Anterior gonapophyses (Fig. 39) triangular, not produced, unpigmented, imperfectly separated from sternite 8. Genital fork (Fig. 38) broad, lightly sclerotized; lateral arms short; stem directed anteriorly in median ventral line, apex extends to mid area of sternite 7. Paraprocts (Figs 37, 40)




**FIGS 22-33**--*Austrosimulium tillyardianum*, female. (22) Fronto-ocular triangle and eye facets. (23) Antenna. (24) Maxillary palp. (25) Lauterborn's organ (sensory vesicle) of 3rd segment of maxillary palp. (26) Tip of mandible. (27) Tip of maxilla. (28) Posterior border of cibarium. (29) Fore leg. Stippling indicates regions of darker coloration. (30) Mid leg. (31) Hind leg. (32) Second hind tarsal segment and apex of hind basitarsus showing calcipala and pedisulcus. (33) Claw of hind leg.




FIGS 34-41--*Austrosimulium tillyardianum*, female. (34) Thorax, lateral view. (35) Costal vein of wing, showing spines, macrotrichia and microtrichia. (36) Abdomen, dorsal view, showing shape of tergites. T1-T9, tergites 1-9. (37) Terminalia, lateral view. T7-T9, tergites 7-9, S7 and S8, sternites 7 and 8. (38) Genital fork, dorsal view. (39) Anterior gonapophyses, ventral view. (40) Paraproct and cercus, ventrolateral view. (41) Cercus, lateral view.

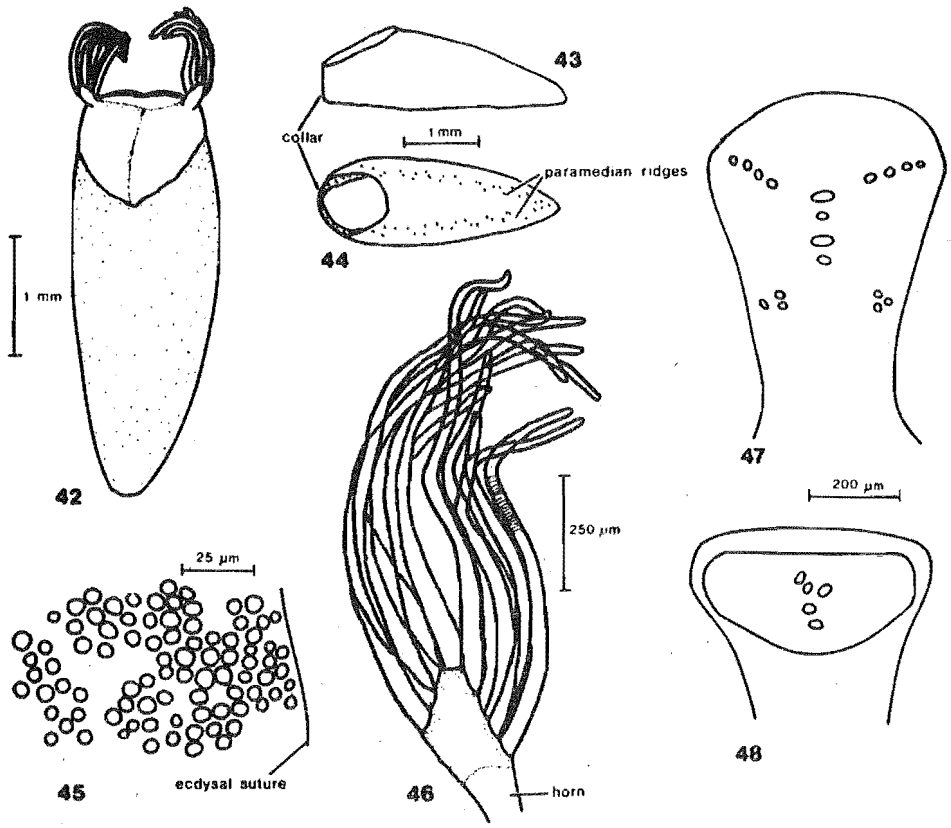
triangular, not joined. Cerci (Figs 40, 41) wider than long, thin; semi-circular in lateral view.

PUPA (Fig. 42): General colour of head and upper thorax dark reddish-brown to brownish-black, lower thorax and legs pale yellowish-brown, abdomen greyish-black to dull black.

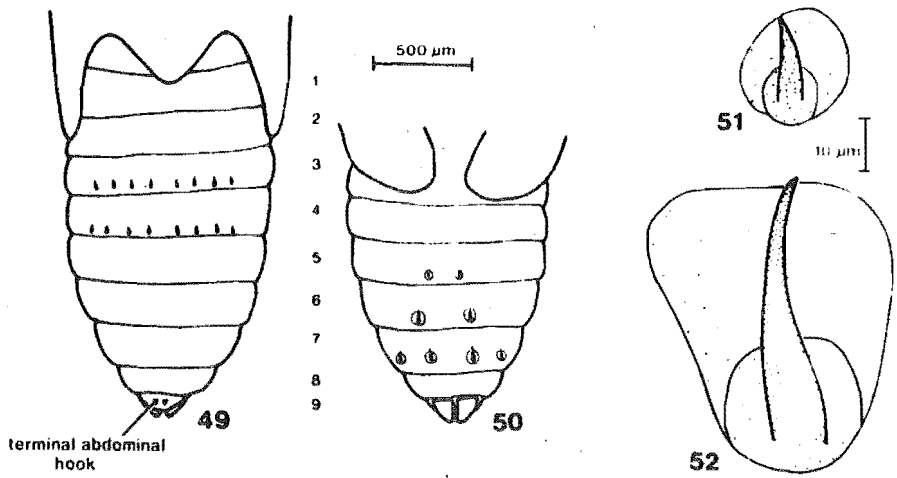
Head: Cephalic apotome of male (Fig. 47) strongly tuberculate on upper half; that of female (Fig. 48) tuberculate laterally only, concave; paler spots present, more in male (Fig. 47). Antennal sheath of male extending 1/2 or slightly more of distance to hind margin of head; antennal sheath of female reaching hind margin of head. Frontal setae absent; facial setae present, 1 on each side between antennal bases; 2 short epicranial setae present on margin of cephalic apotome and antennal sheath at mid length; postorbital spine absent.

Thorax: Dorsum coarsely granulated; tubercles raised, circular, distinct, contiguous (Fig. 45); tubercles form trident-shaped pattern, median line (along ecdysal suture), posterior transverse line and 2 lateral longitudinal lines arising on each end of posterior line (Dumbleton, 1973:Fig. 56), between lines and in pleural areas, tubercles are aggregated in groups of 12 or more. Setae simple, small and difficult to see, sometimes apparently absent, distributed as in Dumbleton (1973). Gill (Fig. 46): Horn short, about 2 1/2 times as long as its greatest breadth; basal 1/3 light yellow, distal 2/3 black, covered with trabeculae, tips anteriorly directed, trabeculae longest distally. Filaments 15-20, light yellow, about 3-4 times the length of horn. Filaments arising from apex and apical 1/3 of external margin, and from basal 1/3 to apex of internal margin; thick, stiff, tapering, inward curving, of reticulated type.

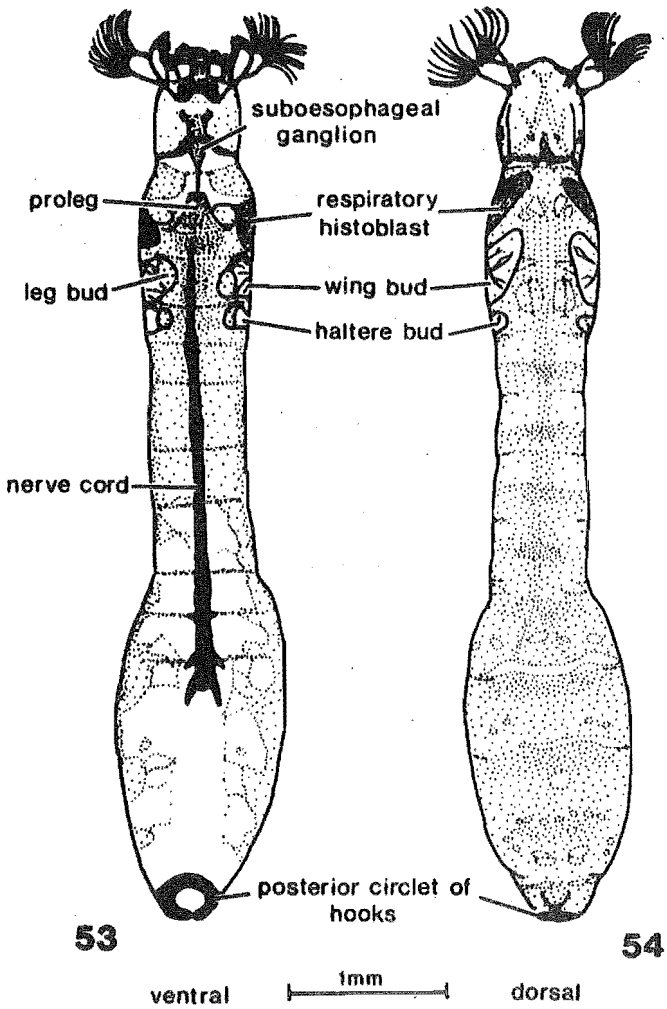
Abdomen: Cuticle pale and membranous; brown lightly sclerotized plates on tergites 1-4 only; no sternite or pleural plates. Onchotaxy (Figs 49, 50), hooks anteriorly directed, on posterior borders of segments; tergites 3 and 4 with 4 small hooks on each side (Fig. 51); sternite 5 with 1 small fine hook each side; sternite 6 with 1 large stout hook each side (Fig. 52); sternite 7 with 2 stout hooks each side, lateral hook smaller. Tergite 9 with 2 small conical horns, representing terminal abdominal hooks (Fig. 49), sternite 9 lacking anchor hairs. Spine combs absent; but some hair-like setae anterolaterally on tergites 5-8, and sternites 3-7 anteromedially.




**FIGS 42-48--*Austrosimulium tillyardianum*, pupa.** (42) Pupa in cocoon, dorsal view. Note inward curving of the respiratory filaments of the pupal gill. (43) Cocoon in profile (shoe-shaped). (44) Cocoon, dorsal view. Note ill-defined paramedian ridges. (45) Left side of thoracic notum showing arrangement of tubercles. (46) Left pupal gill, dorsal view. (47) Cephalic apotome, male. (48) Cephalic apotome, female.




**FIGS 49-52**--*Austrosimulium tillyardianum*, pupa. (49) Abdomen, dorsal surface, showing arrangement of anteriorly directed hooks and weakly developed erect terminal abdominal hooks. (50) Abdomen, ventral view, showing arrangement of anteriorly directed hooks. (51) Anteriorly directed hook of dorsal surface, from segment 4. (52) Anteriorly directed hook of ventral surface, from segment 7.




FIGS 53-54--*Austrosimulium tillyardianum*, 9th instar larva. Body shape and pigmentation pattern. (53) Ventral view. (54) Dorsal view.

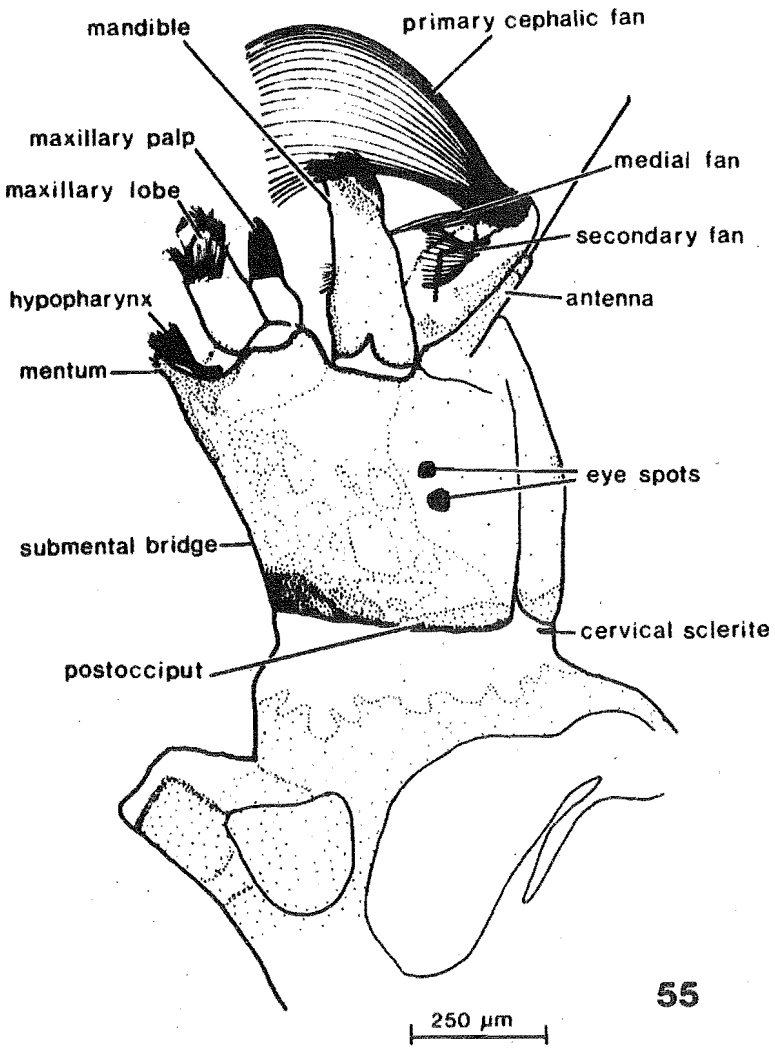


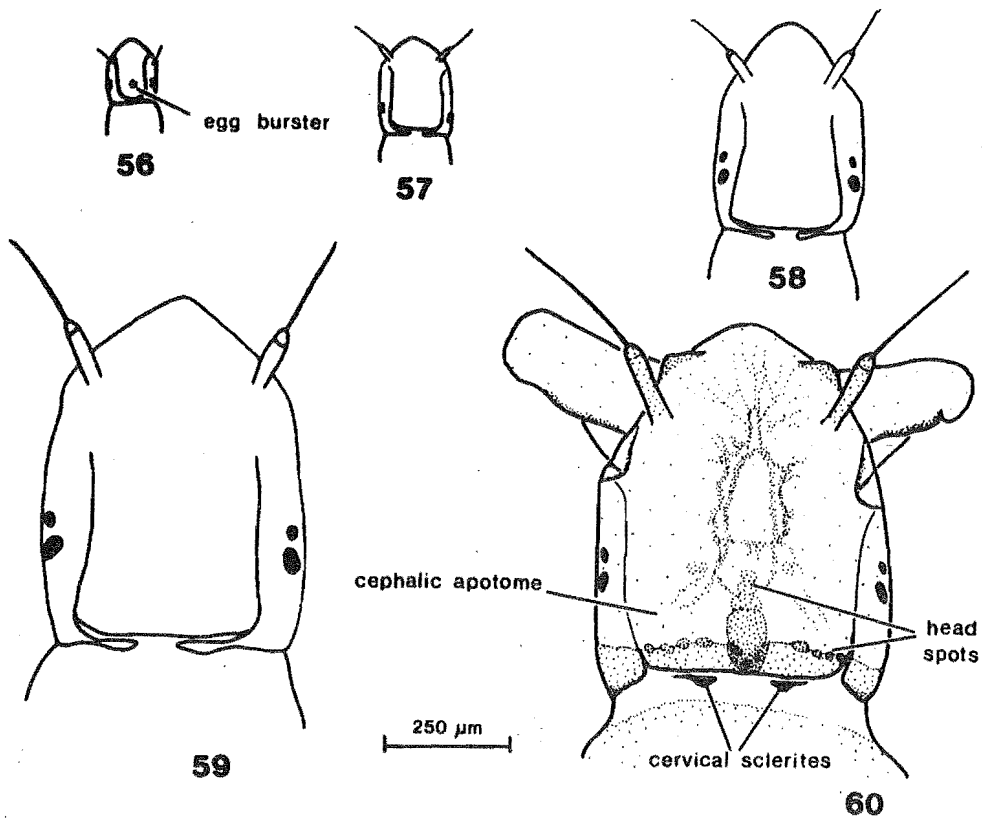

FIG. 55--*Austrosimulium tillyardianum*, 9th instar larva. Lateral view of head.

Cocoon (Figs 42-44): Shoe-shaped, light yellowish-brown, oval, close-fitting to pupa, closely woven, thin, with high collar; posterior margin of aperture irregular; collar margin passing immediately below gills and covering head; posterior 1/4 with floor, loosely woven; 2 ill-defined paramedian ridges dorsolaterally, arising from distal edge of aperture and converge near posterior of cocoon.

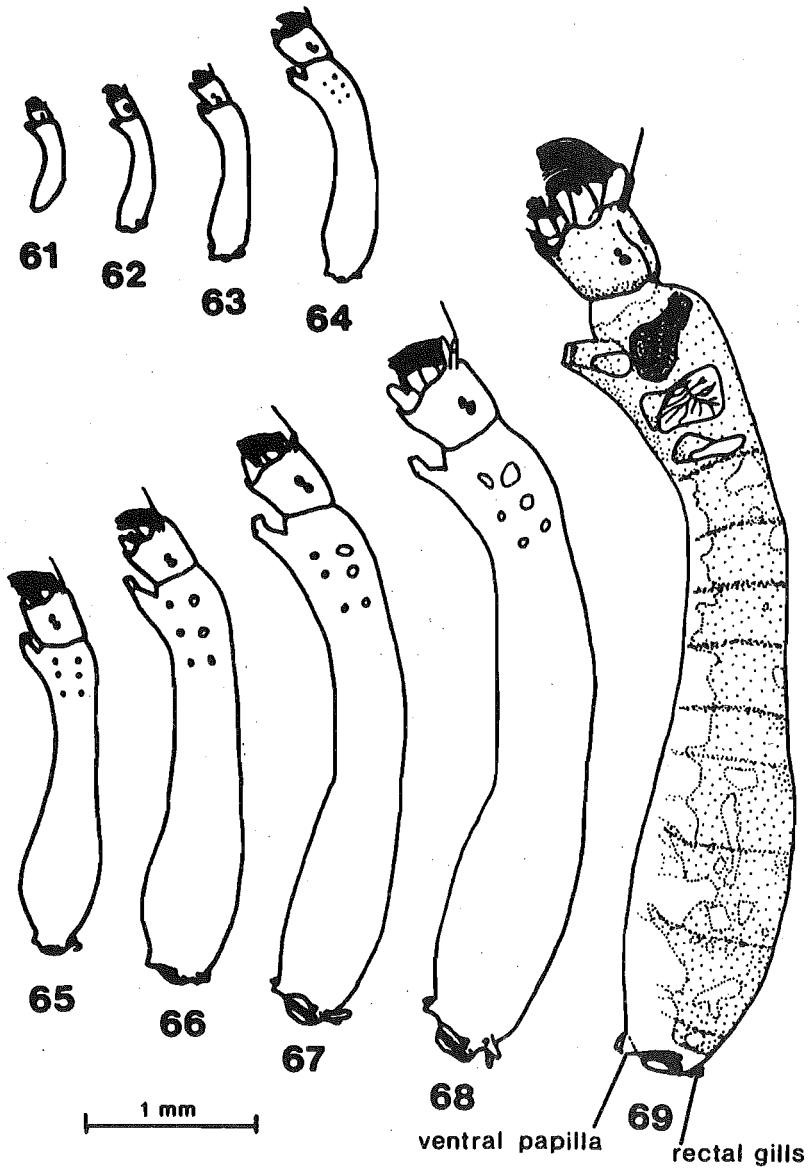
GENERALIZED DESCRIPTION OF LARVA; (Detailed description of each instar follows this generalized description which is based on the 9th instar larva).

General colour: head capsule shiny pale-yellow to light reddish-brown, light greyish-brown dorsal, lateral and anterior ventral surfaces, whitish-transparent posterior ventral surface, nerve cord black; pigmentation patterns as in Figs 53, 54, 69. Body shape (Figs 53, 54, 69); abdomen slightly clubbed with posterior segments clearly set off from anterior segments, thorax somewhat swollen.

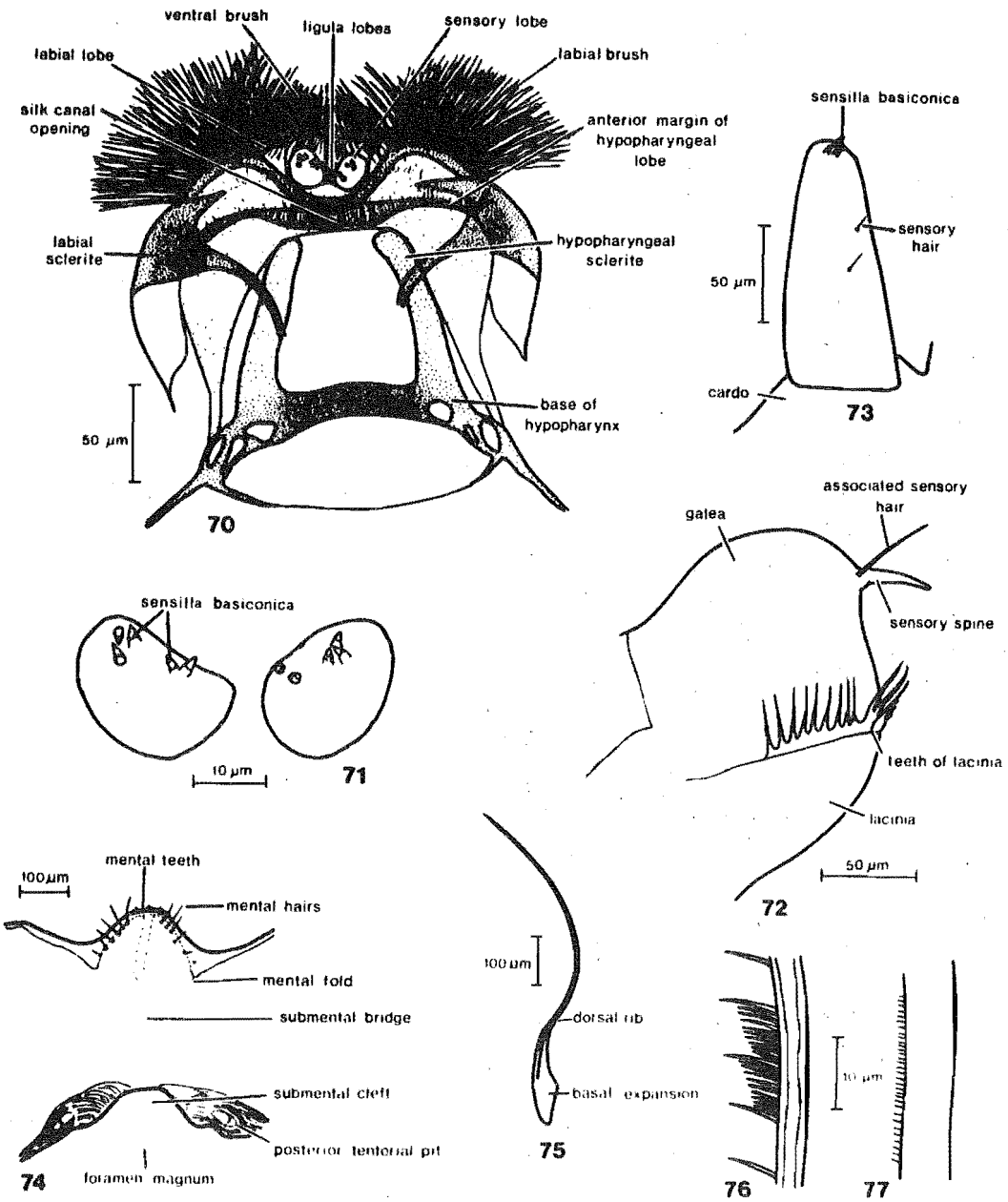
Head: Sides convex (Fig. 60); cephalic apotome widest posteriorly with rounded posterolateral sides (Fig. 60); head spots positive, slightly darker brown than cephalic apotome but poorly demarcated (Fig. 60); 2 well marked black lateral eye spots (Fig. 55), anterior smaller; no eyebrow stripe dorsad of eye spots; postoccipital ring black; cervical sclerites (Fig. 60) black, transverse triangular, separated from postocciput. Submental cleft (Fig. 74) rounded apically, small, 1/2 length of submental bridge; large oval posterior tentorial pit either side. Suboesophageal ganglion (Fig. 53) pigmented, grey-black. Mentum (Figs 74, 109) with 13 teeth, all but median and corner teeth concealed by ventral anterior extension of mentum, teeth black; single row of mental setae about parallel with each lateral side, simple. Antenna 4-segmented (Fig. 90); about 1.5-2.0 times as long as stem of cephalic fan; segments 1 and 2 darker than cephalic apotome, segments 3 and 4 pale; segment 3 sclerotized, especially on apical 1/4 and around mandibular articulation; apical teeth (Fig. 100) stout, third largest; third comb tooth not conspicuously enlarged; other inner teeth well developed, regularly decreasing in size, tips pointed; 2 mandibular serrations, no supernumerary serrations. Maxillary palp (Fig. 73) about 2 1/4 times as long as its basal width; outer dorsal hair tuft of cardo small and inconspicuous; teeth of lacinia as in Fig. 73. Hypopharynx (Fig. 70) normal; sensilla basiconica (Fig. 71) on each sensory lobe arranged in 2 groups of 2 inner and 3 outer sensilla. Cephalic fans (Figs 55, 75-77) normal, concave margin of rays with microtrichia of 2 sizes; secondary fan small, ray tips forming an arc; medial fan very inconspicuous.



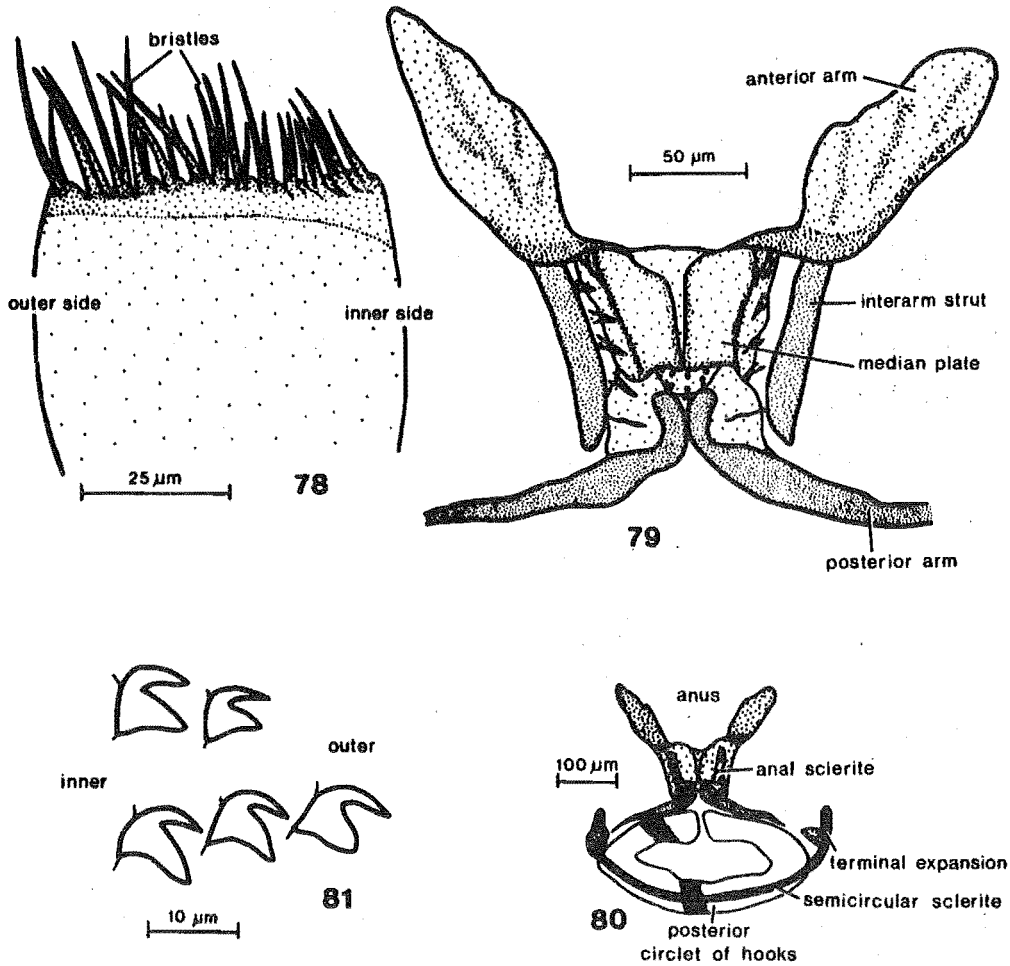

FIGS 56-60--*Austrosimulium tillyardianum* larvae, head region in dorsal view. Only the pigmentation pattern for instar 9 has been indicated, earlier instars are similar. (56) Instar 1. Note that the ends of the postoccipital collar are contiguous. (57) Instar 3. Ends of the postoccipital collar separated. (58) Instar 6. (59) Instar 8. Cervical sclerites still joined to postocciput. (60) Instar 9. Cervical sclerites separated from postocciput.



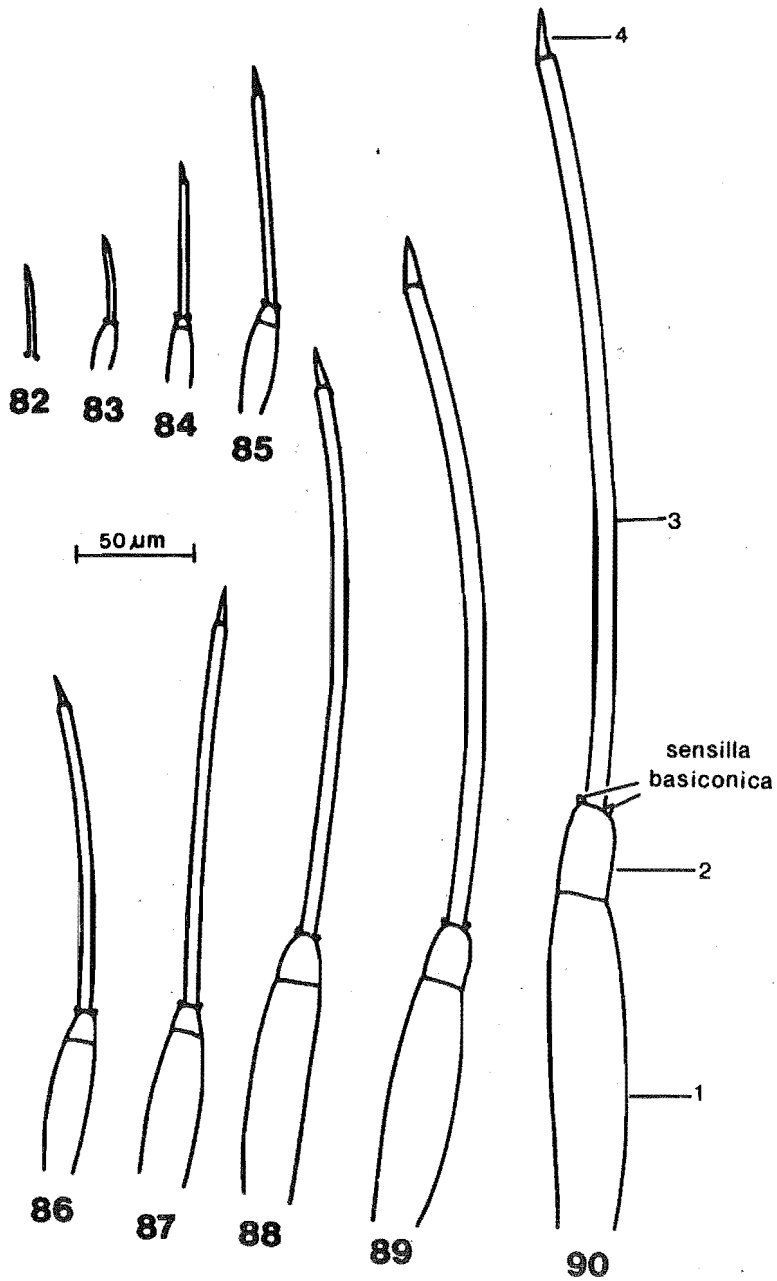

FIGS 61-69--Comparative size of instars of *Austrosimulium tillyardianum* larvae, lateral view. Only the pigmentation pattern of instar 9 has been indicated, earlier instars are similar. (61) Instar 1. (62) Instar 2. (63) Instar 3. (64) Instar 4. Note appearance of imaginal buds. (65) Instar 5. (66) Instar 6. (67) Instar 7. (68) Instar 8. (69) Instar 9.



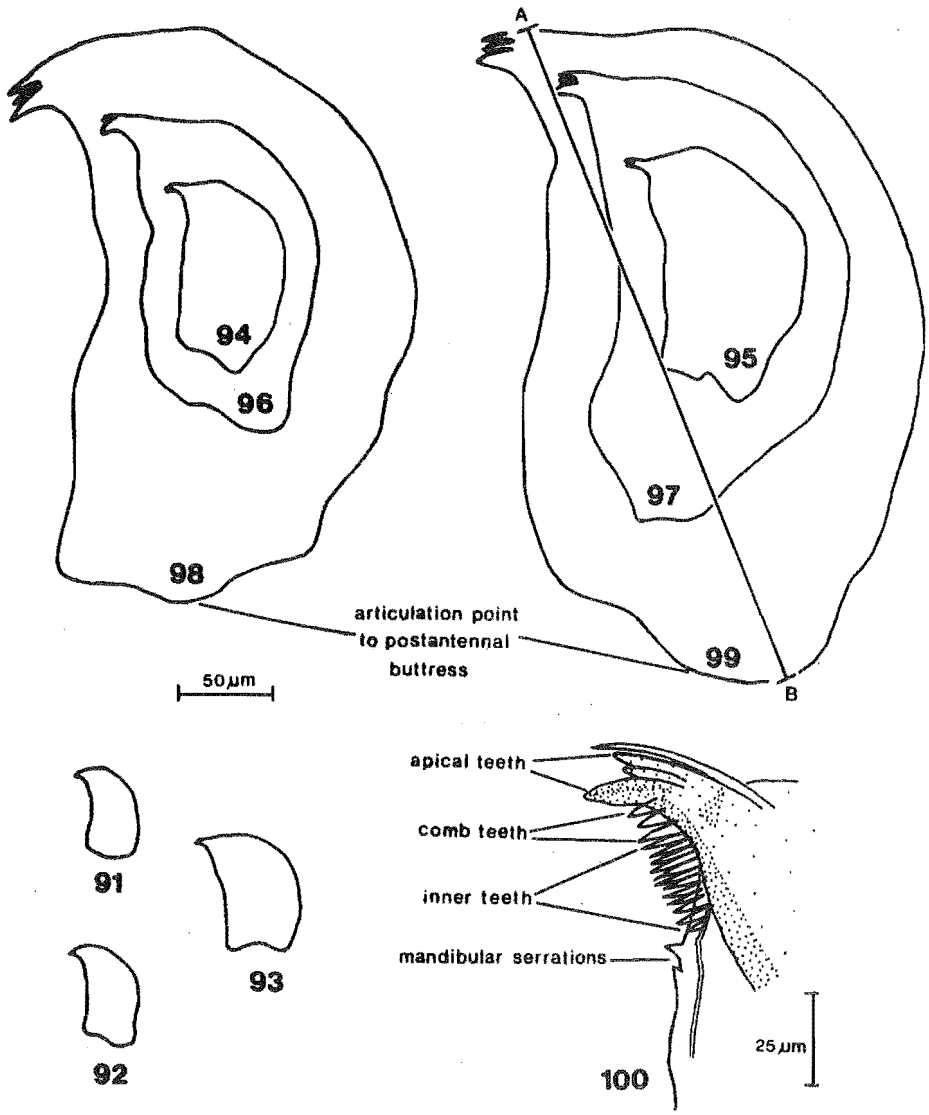

FIGS 70-77--*Austrosimulium tillyardianum*, 9th instar larva. (70) Labiohypopharyngeal complex, dorsal view. Opening of silk canal is medially located between hypopharyngeal lobe and labial lobe. (71) Sensory lobes, showing grouping of sensilla basiconica. (72) Maxillary lobe, dorsal view. (73) Maxillary palp, dorsal view. (74) Head capsule, ventral view. (75) Primary ray of cephalic fan. (76) Middle section of primary ray showing pattern of microtrichia. (77) Basal section of primary ray showing pattern of microtrichia.



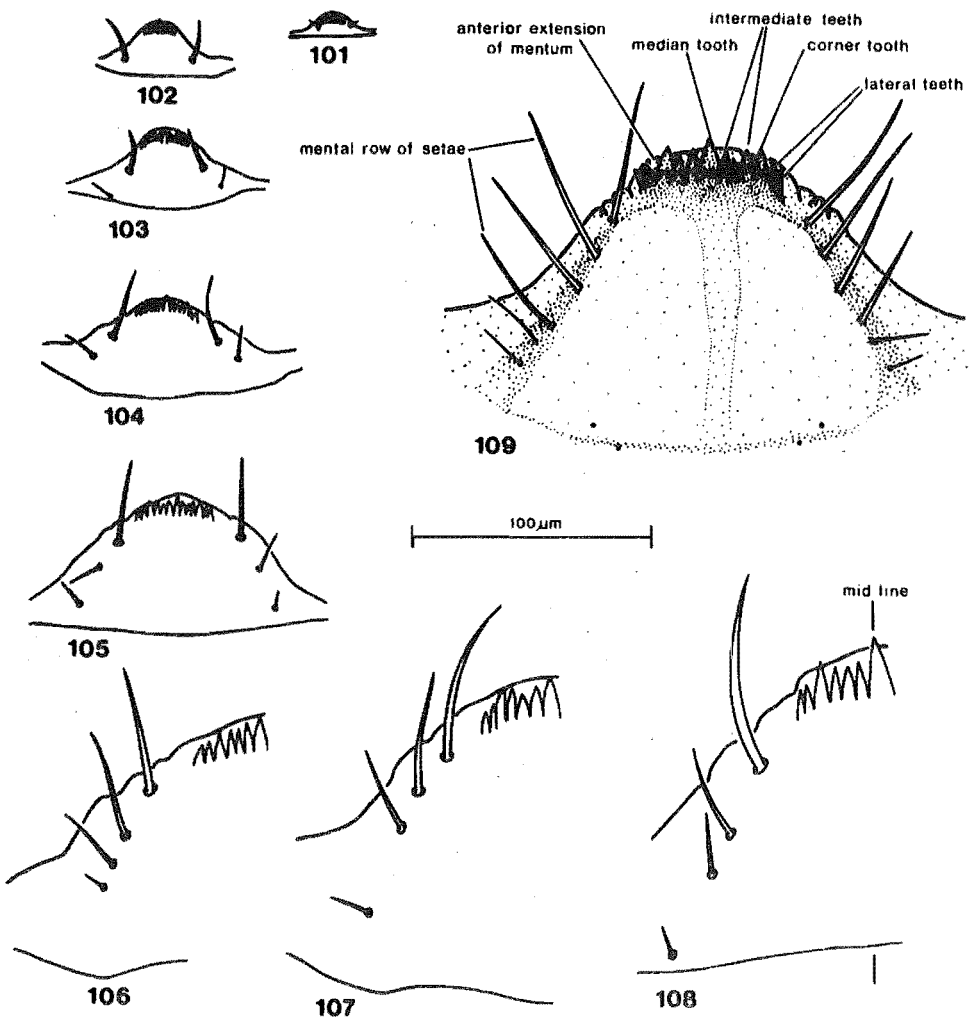

FIGS 78-81--*Austrosimulium tillyardianum*, 9th instar larva. (78) Lateral sclerite of proleg. Bristles in groups of 2, rarely 3. (79) Anal sclerite. (80) Anal sclerite, semicircular sclerite, and posterior circlet of hooks. (81) Outward-facing hooks of posterior circlet.



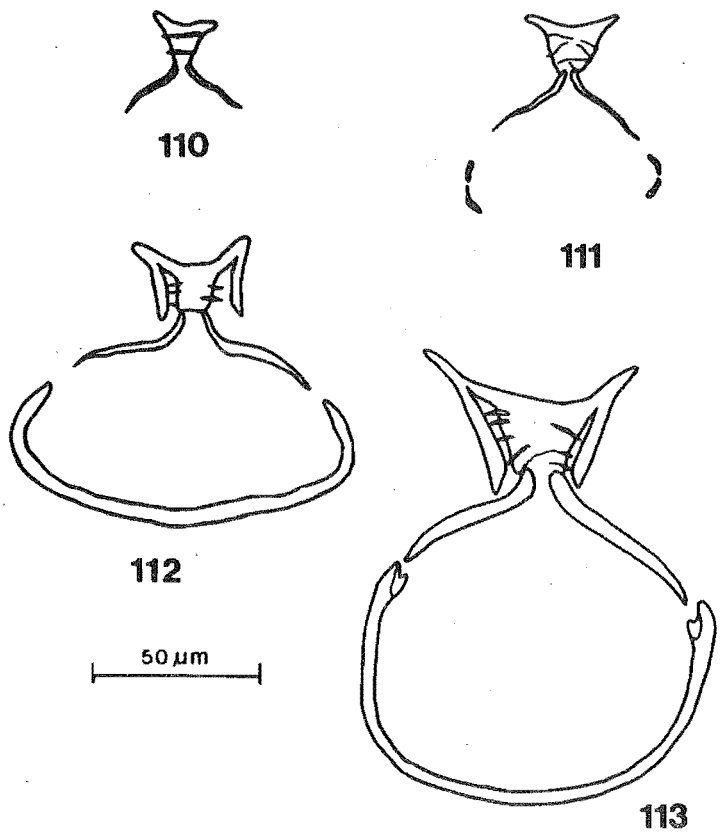

FIGS 82-90--*Austrosimulium tillyardianum*. Larval antennae. (82) Instar 1, 2-segmented. (83) Instar 2, 3-segmented. (84) Instar 3, 4-segmented. (85) Instar 4. (86) Instar 5. (87) Instar 6. (88) Instar 7. (89) Instar 8. (90) Instar 9.




FIGS 91-100--*Austrosimulium tillyardianum*. Comparative size of larval mandibles. (91) Instar 1. (92) Instar 2. (93) Instar 3. (94) Instar 4. (95) Instar 5. (96) Instar 6. (97) Instar 7. (98) Instar 8. (99) Instar 9. AB, length measured. (100) Tip of mandible, instar 9, showing arrangement of teeth and mandibular serrations.




FIGS 101-109--Mental plates of *Austrosimulium tillyardianum* larvae. (101) Instar 1, 9 mental teeth. (102) Instar 2, 13 mental teeth. (103) Instar 3. (104) Instar 4. (105) Instar 5. (106) Instar 6. (107) Instar 7. (108) Instar 8. (109) Instar 9. Note that one of the intermediate teeth on the left side is missing.




FIGS 110-113--*Austrosimulium tillyardianum*. Development of anal and semicircular sclerites of larvae. (110) Instar 2. Anal sclerite only present. (111) Instar 3. Anal sclerite and beginning of semicircular sclerite. (112) Instar 4. Complete semicircular sclerite, interarm struts of anal sclerite present. (113) Instar 5. Terminal expansions on semicircular sclerite present.

Thorax: Cuticle bare. Spiracular scars faint. Lateral sclerite (Fig. 78) of proleg lightly sclerotized, anterior margin with lobed appearance; about 25 bristles, arranged in groups of 2, sometimes 3, longest on outer (ventral-most) side.

Abdomen: Cuticle bare. Spiracular scars very inconspicuous. 2 subconical ventral papillae present posteriorly (Fig. 69). Rectal gills simple (Fig. 69), milky white without pattern, 3 lobes, each bluntly rounded. Accessory sclerites and rectal scales absent. Anal sclerite (Figs 79, 80) X-shaped; anterior arms broader than posterior arms; apices of anterior arms bluntly pointed, apices of posterior arms sharply pointed; anterior margin of anterior arms lightly sclerotized; interarm struts arise from apical 1/3 of anterior arms, run ventral to but lateral of median plate to near base of posterior arm, do not fuse to posterior arms; posterior margin of median plate with about 9 tubercles. Semicircular sclerite (Fig. 80) stout, with terminal expansions. Hooks of posterior circlet of hooks (Fig. 81) normal.

INSTAR 1 (Fig. 61)

Mean preserved length: 0.52 mm.
Egg burster (Fig. 56): present on cephalic apotome, in a median line, level with eye spots. Consists of a raised point surrounded by 7 small points, black. This is absent in instar 2.
Antenna (Fig. 82): 2-segmented. Mean total length: 43.8 µm.
Cephalic rays: about 10 primary and 8 secondary rays, weakly sclerotized.
Mandible (Fig. 91): mean length AB: 51.5 µm. 3 apical teeth, 2-3 comb teeth. All subsequent instars have 3 apical teeth.
Maxillary palp: weakly sclerotized, nearly transparent.
Mentum (Fig. 101): 9 mental teeth; in 3 groups of 3, central tooth of median group more anterior than others. 1 seta in each mental row.
Cervical sclerites (Fig. 56): not developed, ends of postoccipital collar contiguous.
Proleg circlet of hooks: 12 rows with 1 hook per row.
Posterior circlet of hooks: about 45 rows with 2-3 hooks per row.
Anal sclerite: not developed.
Semicircular sclerite: not developed.

INSTAR 2 (Fig. 62)

Mean preserved length: 0.75 mm.
Antenna (Fig. 83): 3-segmented. Mean total length: 68.9 µm.

Cephalic rays: about 32 primary and 9 secondary rays, moderately sclerotized.
Mandible (Fig. 92): mean length AB: 60.1 µm. 3 comb teeth. All subsequent instars have 3 comb teeth.
Maxillary palp: moderately sclerotized.
Mentum (Fig. 102): 13 mental teeth as in final instar, median one most anterior. 1 seta in each mental row.
Cervical sclerites: not developed, slight separation of ends of postoccipital collar, as in Fig. 57.
Proleg circlet of hooks: 12 rows with 1-2 hooks per row.
Posterior circlet of hooks: about 50 rows with 3-4 hooks per row.
Anal sclerite (Fig. 110): present, backwardly directed interarm strut from each anterior arm absent.
Semicircular sclerite: not developed.

INSTAR 3 (Fig. 63)

Mean preserved length: 0.99 mm.
Antenna (Fig. 84): 4-segmented, as are all subsequent instars. Mean total length: 110.2 µm.
Cephalic rays: about 21-25 primary and 11 secondary rays; strongly sclerotized, similar to all subsequent instars.
Mandible (Fig. 93): mean length AB: 76.2 µm. 1-2 inner teeth.
Maxillary palp: strongly sclerotized, as are all subsequent instars.
Mentum (Fig. 103): 2 setae in each mental row, posterior seta shorter than anterior seta.
Cervical sclerites (Fig. 57): not developed, increasing separation of ends of postoccipital collar.
Proleg circlet of hooks: about 14 rows with 2 hooks per row.
Posterior circlet of hooks: about 50-55 rows with 5-6 hooks per row.
Anal sclerite (Fig. 111): present, interarm strut from each anterior arm absent.
Semicircular sclerite (Fig. 111): either not developed, or partly developed.

INSTAR 4 (Fig. 64)

Mean preserved length: 1.32 mm.
Antenna (Fig. 85): mean total length: 139.5 µm.
Cephalic rays: about 26-30 primary and 12 secondary rays.
Mandible (Fig. 94): mean length AB: 105.3 µm. 2-5 inner teeth.

Mentum (Fig. 104): 2, or sometimes 3, setae in each mental row; the posterior seta shorter than anterior seta(-e).
Cervical sclerites: not developed, ends of the postoccipital collar drawing apart, as in Fig. 57.
Imaginal buds (Fig. 64): all present (pupal respiratory histoblast, adult wing bud, haltere bud and 3 leg buds), just visible as clear patches against pigmented thorax. No differentiation of tissue within buds. Prothoracic leg bud sometimes absent.
Proleg circlet of hooks: about 14 rows with 4 hooks per row.
Posterior circlet of hooks: about 52-60 rows with 6-7 hooks per row.
Anal sclerite (Fig. 112): present, interarm strut from each anterior arm present but weakly sclerotized.
Semicircular sclerite (Fig. 112): developed, but without terminal expansions.

INSTAR 5 (Fig. 65)

Mean preserved length: 1.81 mm.
Antenna (Fig. 86): mean total length: 179.4 µm.
Cephalic rays: about 30-34 primary and 13 secondary rays.
Mandible (Fig. 95): mean length AB: 135.3 µm. 3-7 inner teeth.
Mentum (Fig. 105): 3, or sometimes 4, setae in each mental row; posterior 2 setae shorter than anterior seta(-e).
Cervical sclerites: not developed, postoccipital collar as in Fig. 58.
Imaginal buds (Fig. 65): all present, no differentiation of tissue within buds.
Proleg circlet of hooks: about 17-18 rows with 4-6 hooks per row.
Posterior circlet of hooks: about 63-67 rows with 6-8 hooks per row.
Anal sclerite (Fig. 113): present, interarm strut strongly sclerotized, similar to all subsequent instars.
Semicircular sclerite (Fig. 113): developed, with terminal expansions, width of an expansion no more than twice width of rest of sclerite, shallow notch to expansion.

INSTAR 6 (Fig. 66)

Mean preserved length: 2.64 mm.
Antenna (Fig. 87): mean total length: 231.3 µm.
Cephalic rays: about 32-35 primary and 15 secondary rays.
Mandible (Fig. 96): mean length AB: 177.6 µm. 5-9 inner teeth.
Mentum (Fig. 106): 3-4 setae in each mental row, posterior 2 setae shorter than anterior seta(-e).

Cervical sclerites (Fig. 58): slightly differentiated from ends of postoccipital collar.

Imaginal buds (Fig. 66): pupal respiratory histoblast and adult wing bud slightly larger than other buds, no differentiation of tissue within buds.

Proleg circlet of hooks: about 22-25 rows with 5-7 hooks per row.

Posterior circlet of hooks: about 78-88 rows with 9-12 hooks per row.

Semicircular sclerite: width of terminal expansions about 3 times width of rest of sclerite. Sides of expansions more heavily sclerotized than centre, notch about 1/3 length of expansion.

INSTAR 7 (Fig. 67)

Mean preserved length: 3.54 mm.

Antenna (Fig. 88): mean total length: 289.4 µm.

Cephalic rays: about 35-44 primary and 14-16 secondary rays.

Mandible (Fig. 97): mean length AB: 225.4 µm. 6-9 inner teeth.

Mentum (Fig. 107): 4-5 setae in each mental row, posterior 2 setae shorter than anterior setae.

Cervical sclerites: slightly differentiated from ends of postoccipital collar, as in Fig. 58.

Imaginal buds (Fig. 67): diameter of pupal respiratory histoblast and adult wing bud twice that of other buds, no differentiation of tissue within buds.

Proleg circlet of hooks: about 24-25 rows with 7-9 hooks per row.

Posterior circlet of hooks: about 85-90 rows with 11-12 hooks per row.

Semicircular sclerite: notch of terminal expansion about 1/2 its length, as in all subsequent instars.

INSTAR 8 (Fig. 68)

Mean preserved length: 4.21 mm.

Antenna (Fig. 89): mean total length: 329.8 µm.

Cephalic rays: about 40-45 primary and 15-16 secondary rays.

Mandible (Fig. 98): mean length AB: 279.4 µm. 7-11 inner teeth.

Mentum (Fig. 108): 4-5 sometimes 6 setae in each mental row, posterior 2 setae shorter than anterior setae.

Cervical sclerites (Fig. 59): differentiated, but still attached to ends of postoccipital collar; moderately sclerotized.

Imaginal buds (Fig. 68): diameter of pupal respiratory histoblast and adult wing bud twice that of other buds, no differentiation of tissue within buds.

Proleg circlet of hooks: about 25-27 rows with 8-10 hooks per row.
Posterior circlet of hooks: about 90-105 rows with 12-15 hooks per row.

INSTAR 9 the pharate pupa (Hinton, 1958) (Figs 53, 54, 69)

Mean preserved length: 5.28 mm.
Antenna (Fig. 90): mean total length: 411.0 µm.
Mandible (Fig. 99): mean length AB: 350.1 µm. 9-12 inner teeth (Fig. 100).
Mentum (Fig. 109): 5-7 setae in each mental row, posterior 2 setae shorter than anterior setae. Sometimes 1 less seta in 1 row than in other.
Cervical sclerites (Fig. 60): separated from ends of postoccipital collar; strongly sclerotized.
Imaginal buds (Fig. 69): pupal respiratory histoblast with folded filaments white in early stage, but black just before pupation. Wing and haltere buds with a venation pattern taking form of pigmented lines, appearing as instar matures. Leg buds with inflexions present.
Proleg circlet of hooks: about 27-32 rows with 9-12 hooks per row.
Posterior circlet of hooks: about 100-120 rows with 14-18 hooks per row (Fig. 81).

EGG: Normal simuliid type; triangular-ovoid, asymmetrical because of bulge on one side. Surface smooth. Whitish-yellow when laid, dark yellowish-brown prior to hatching. Laid on downstream surface of rocks 15-100 mm below water level, 250-330 eggs in egg mass 6-8 mm by 3-5 mm, single layer, fixed to stone by transparent gelatinous material.

MATERIAL EXAMINED

(L = larvae, P = pupae, A = adults; T.K.C. = T.K. Crosby)

Type material pinned; specimens collected during study preserved in 90 °/₀ ethanol, some adults pinned.

Holotype male, Maitai River, Nelson, -vi.23, bred, A.L. Tonnoir; allotype female, same data; paratypes, 2 males, 3 females, same data; 2 males, Nelson, -i.22, bred, A.L. Tonnoir: all in Entomology Division, D.S.I.R., Auckland.

53 vials containing specimens collected by Dumbleton, preserved in alcohol: all in Entomology Division, D.S.I.R., Auckland. (Distribution given in Dumbleton (1973:Fig. 4)).

Maitai River, Nelson (type locality), 41°18'S, 173°19'E, 6.viii.72, T.K.C. & Z.J. Mazur, LPA. Cold Stream, 42°40'S, 173°19'E, 4.xi.69, T.K.C., LP. Cave Stream, Broken River Ski Field Entrance, 43°09'S, 171°44'E, 2.ii.70, T.K.C., L. Cave Stream, cave exit, 43°12'S, 171°44'E, 5.iii.70, T.K.C., LPA. Broken River, bridge, 43°12'S, 171°44'E, 5.iii.70, T.K.C., P. Kowai River, 43°19'S, 171°47'E, 2.ii.70, 5.iii.70, T.K.C., LP. Stream near The Spectacles, 43°20'S, 171°35'E, -vii.70, W.J. Crumpton, LP. South Branch, Shipley's farm, 43°28'S, 172°34'E, 1.ix.69, T.K.C., L. Kaituna Valley Stream, 43°44'S, 172°43'E, 14.vi.69, T.K.C., LP. Prices Valley Stream, 43°46'S, 172°43'E, 14.vi.69, T.K.C., LPA. Stream crossing Puaha Road, 43°45'S, 172°51'E, 3.vii.69, 7.vii.69, 26.viii.69, T.K.C., L. Opuahau Stream, Little River, 43°46'S, 172°49'E, 3.vii.69, 26.viii.69, 19.vii.70, T.K.C., LP. Wainui Valley Stream, 43°49'S, 172°54'E, 31.vii.69, 26.viii.69, 9.x.69, 1.xi.69, 25.xi.69, 27.i.70, 19.vii.70, 30.viii.70, 29.ix.70, 18.x.70, 23.i.71, 13.ii.71, 14.iii.71, 3.iv.71, 25.iv.71, 2.v.71, 9.v.71, 16.v.71, 30.v.71, 6.vi.71, 13.vi.71, 20.vi.71, 27.vi.71, 4.vii.71, 11.vii.71, 18.vii.71, 25.vii.71, 1.viii.71, 8.viii.71, 15.viii.71, 22.viii.71, 29.viii.71, 5.ix.71, 12.ix.71, 19.ix.71, 26.ix.71, 3.x.71, 10.x.71, 17.x.71, 24.x.71, 31.x.71, 7.xi.71, 14.xi.71, 21.xi.71, 19.xii.71, 16.i.72, T.K.C., LPA. Opihi River, 44°05'S, 170°40'E, 23.i.71, A.M. Fallis, LP.

## DISCUSSION

### Distribution of *A. (A.) tillyardianum*

*Austrosimulium tillyardianum* is a common species found in both the North and South Islands (Dumbleton, 1973). The larvae are characteristically found in open-country lowland streams, and the stones to which the larvae attach are usually rounded and free of algae. Often other *Austrosimulium* species are associated with *tillyardianum*; these include *australense*, *laticorne* Tonnoir, *longicorne* Tonnoir, and *multicorne* Tonnoir.

*A. tillyardianum* was the only simuliid found in the Wainui Valley Stream in the entire study period (June 1969 to January 1973), although Dumbleton (1973) also recorded *longicorne* as being present in the Wainui. It appears likely that Dumbleton either inadvertently misidentified *tillyardianum*, or he incorrectly referred to the Wainui in the Marlborough-Canterbury region instead of to one of the Wainui's of the North Auckland region (*longicorne* is found in North Auckland).

## Relationship of *A. (A.) tillyardianum* with other New Zealand species

*A. tillyardianum* is a member of the endemic *australense* species group (Dumbleton, 1973). The seven species of the group are divided into two subgroups: the *australense* subgroup containing two New Zealand-wide species *australense* and *longicorne*; and the *tillyardianum* subgroup containing five species (*tillyardianum*, *m. multicorne* and *m. fiordense* Dumbleton, *stewartense* Dumbleton, *l. laticorne* and *l. alveolatum* Dumbleton, and *albovelatum* Dumbleton) with restricted distributions in either the North or South Islands. *A. tillyardianum* is considered to be closest related to *multicorne* (Dumbleton, 1973).

Since the similarities and differences between *tillyardianum* and the other New Zealand species have been described and illustrated recently by Dumbleton (1973), they will not be repeated here. Some observations and interpretations given by Dumbleton (1973) differ from those made on *tillyardianum* in the present study, however, and these will now be discussed.

1. The mandible of the first instar larva of *tillyardianum* possesses three apical and two or three comb teeth. In his general description of a first instar *Austrosimulium* larva Dumbleton (1973) stated that there are ". . . two apical and two subapical paler teeth" present. Since this statement appears to be based on an examination of one species only (probably *stewartense*) (Dumbleton, 1964), this number of teeth can not be regarded as a generic characteristic.

2. Under the heading "subsequent instars", meaning from the third instar, Dumbleton (1973) mentions that the interarm struts of the anal sclerite and the semicircular sclerite around the posterior circlet of hooks are present. In *tillyardianum*, however, the interarm struts do not appear until the fourth instar. Also, whereas the semicircular sclerite may be partly developed in the third instar, it is never fully developed until the fourth instar.

3. The interpretation of the simuliid wing venation presented by Dumbleton (1973) differs from the generally accepted interpretation (Crosskey,1969). The faint submedian fork is called the $M_{3+4}$ by Dumbleton, but I have shown elsewhere that it is not a true vein (Crosby, in press). The equally faint *1A* vein is omitted from Dumbleton's figure, and the well

defined *2A* vein is labelled as the only anal vein.

4. In many pupae of *tillyardianum* from the Wainui Valley Stream, the setae located on the head and thorax were extremely difficult to see, and, at times, appeared to be absent. This suggests that the comparatively isolated Wainui population has some slight differences from the populations of *tillyardianum* examined by Dumbleton (1973).

## ACKNOWLEDGMENTS

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Paper 2

# Critique of instar determination in Simuliidae (Diptera), with particular reference to methods applied to a study of *Austrosimulium tillyardianum*

Biometrical evidence is presented to demonstrate that there are nine larval instars in the life history of Austrosimulium tillyardianum. Four methods of analyzing groups formed from measurements are examined. Previous simuliid instar determination studies are evaluated.

Cautionary quote:

"Kehoe's Law"

"If you put garbage in one end you are going to get garbage out of the other end, no matter how sophisticated your research design or statistical manipulation."

Powles (1968) A Martian psychiatrist views with alarm the current state of the term "Schizophrenia". Diseases of the Nervous System, Supplement 29 : 5-10.

Contents

## Introduction

Only recently have detailed instar determination studies been undertaken on the Simuliidae, and attempts made to define larval instars by both morphological characteristics and measurements. Studies before 1957, summarized by Grenier & Feraud (1960) and Johnson & Pengelly (1970), usually reported that simuliids possessed six larval instars, based on morphological differences and size differences of structures. Later, more detailed studies have indicated that simuliid species have six or seven larval instars (Table I), although two simuliid species were believed to have only four larval instars (Yakuba, 1960) and one species was shown to have eight (Smith, unpublished 1969).

Before I could begin a population study on a common New Zealand simuliid Austrosimulium (Austrosimulium) tillyardianum Dumbleton (Crosby, in prep.), it was necessary to determine the number of larval instars in its life history to allow changes in population structure with time to be followed (Smith, unpublished 1969; Kaćanski, 1970; Halgoš, 1972). A. tillyardianum is a multivoltine species, and in the study area, the Wainui Valley Stream, Canterbury, larvae of all sizes can be found throughout the year.

My investigations showed that nine instars could be recognized morphologically and a description of each instar is given elsewhere (Crosby, in press). The discovery of nine instars for A. tillyardianum was unexpected since it is the highest number recorded for any simuliid species to date, and is probably the highest larval instar number recorded for any nematoceran (Hennig, 1948). The main purpose of this paper, therefore, is to present the biometrical evidence which showed that A. tillyardianum instar groupings constructed on the basis of morphological

TABLE I

Species of Simuliidae in which the number of larval instars has been studied. A) studies primarily concerned with instar determination, B) other, less detailed studies. (+ = used; - = not used)

| | Species | Country | Number of instars | Evidence for separation | | Authority |
|---|---|---|---|---|---|---|
| | | | | Morphological | Biometrical | |
| A | | | | | | |
| | *Prosimulium* (*Prosimulium*) *hirtipes* (Fries) | England | 8 | + | + | Smith (unpublished 1969) |
| | *Simulium* (*Edwardsellum*) *damnosum* Theobald | Upper Volta, Africa | 7 | + | + | Grenier & Feraud (1960) |
| | *Simulium* (*Odagmia*) *kiritshenkoi* Rubzov | Armenian SSR | 6 | + | + | Terteryan (1957) |
| | *Simulium* (*Odagmia*) *nitidifrons* Edwards | England | 6 | + | + | Harrod (1964) |
| | *Simulium* (*Odagmia*) *ornatum* Meigen | England | 6 | + | - | Grant (1961) |
| | *Simulium* (*Odagmia*) *ornatum* Meigen | Yugoslavia | 7 | + | + | Kaćanski (1968) |
| | *Simulium* (*Simulium*) *galeratum* Edwards | Irkutsk Province, USSR | 4 | - | + | Yakuba (1960) |
| | *Simulium* (*Simulium*) *rugglesi* Nicholson & Mickel | Ontario, Canada | 7 | + | + | Johnson & Pengelly (1970) |

| | | | | | |
|---|---|---|---|---|---|
| *Simulium* (*Wilhelmia*) *equinum* (Linnaeus) | Irkutsk Province, USSR | 4 | - | + | Yakuba (1960) |
| *Simulium* (*Wilhelmia*) *paraequinum transcaucasicum* (Rubzov) | Armenian SSR | 6 | + | + | Terteryan (1957) |
| *Austrosimulium* (*Austrosimulium*) *tillyardianum* Dumbleton | New Zealand | 9 | + | + | Present study |
| B | | | | | |
| *Prosimulium* (*Parahelodon*) *decemarticulatum* (Twinn) | Wisconsin, USA | 6 | - | - | Anderson & Dicke (1960) |
| *Prosimulium* (*Prosimulium*) *arvernense* Grenier | Czechoslovakia | 7 | - | + | Halgoš (1972) |
| *Prosimulium* (*Prosimulium*) *hirtipes* (Fries) | West Germany | 7 | - | + | Zwick (unpublished 1971) |
| *Prosimulium* (*Prosimulium*) *mysticum* Peterson | Ontario, Canada | 7 | - | + | Mansingh, Steele & Helson (1972) |
| *Prosimulium* (*Prosimulium*) *toemoesvaryi* (Enderlein) | West Germany | 7 | - | + | Zwick (unpublished 1971) |
| *Simulium* (*Boophthora*) *erythrocephalum* (De Geer) | England | 6 | + | + | Puri (1925) |
| *Simulium* (*Edwardsellum*) *damnosum* Theobald | Zaïre Republic, Africa | 6 | - | - | Wanson & Henrard (1945) |

| | | | | | |
|---|---|---|---|---|---|
| Simulium (Eusimulium) aureum Fries | England | 6 | + | + | Puri (1925) |
| Simulium (Eusimulium) vernum Macquart | England | 6 | + | + | Edwards (1920) |
| Simulium (Odagmia) ornatum Meigen | Yugoslavia | 6 | + | - | Baranov (1926) |
| Simulium (Odagmia) ornatum Meigen | Scotland | 6 (at least) | + | + | Smart (1934) |
| Simulium (Odagmia) ornatum Meigen | Czechoslovakia | 7 | - | + | Jedlička (1972) |
| Simulium (Simulium) arcticum Malloch | Saskatchewan, Canada | 6 | + | + | Cameron (1922) |
| Simulium (Wilhelmia) equinum (Linnaeus) | Yugoslavia | 6 | + | - | Baranov (1926) |
| Simulium (Wilhelmia) equinum (Linnaeus) | Scotland | 6 | - | + | Maitland & Penney (1967) |
| Simulium (Wilhelmia) latipes Meigen | Yugoslavia | 6 | - | - | Živković (1951) |
| Austrosimulium (Austrosimulium) tillyardianum Dumbleton | New Zealand | 4 (at least) | - | - | Tonnoir (1925) |

differences were also biometrically discrete. From such evidence it was possible to conclude that larval instars separated according to morphological characters corresponded to those groupings which could be constructed from measurements of structures.

Four methods used to analyze the measurements are evaluated. The efficiency of these methods purported to "prove" or aid separation of groups is discussed with particular reference to the nine instars found for A. tillyardianum. As a test of the sensitivity of the methods to demonstrate the "correctness" of the instar groupings, some instars were lumped to see if this could be detected, and, if so, whether the true extent of the lumping could be revealed.

Several aspects of this study are exploratory. For example, in the multiple discriminant function analysis section more analyses were undertaken than were required to demonstrate the existence of nine instars. The purpose of this was to investigate how individual larvae within instar groups responded to different combinations of variables as indicated by the resultant discriminant values. The results of such data manipulations proved to be of value in defining the usefulness of the method.

Finally, earlier detailed instar determination studies are evaluated, and the results of some are shown to be capable of being re-interpreted to give a higher number of larval instars.

## A. tillyardianum material

### Source of larvae

Field collected and laboratory reared larvae were used in the study. All larvae were collected from an experimental channel of the Wainui Valley Stream, Banks Peninsula (43° 49'S, 172° 54'E), and were preserved in 90% ethanol within 2-4 h of collection. Laboratory reared larvae were hatched from egg masses also collected from the experimental channel. A. tillyardianum was the only simuliid present in the stream (Crosby, in press).

A total of 342 larvae were studied, and a complete set of 16 variables (p. 52 ) was determined for 289 of them. Each larva in an instar group was assigned a number, and this number is used throughout this account to refer to that individual.

### Collection dates

Three sets of larvae were measured (Table II ) -- approximately equal numbers from each instar. The "standardization" set of larvae were those used to form the initial instar groupings, whereas the "test" sets of larvae were used to test the validity of the instar groupings so constructed. The 3-4-71 test set collected six months after the standardization set also served to check for any seasonal differences. By contrast, the 26-9-71 test set collected one year after the standardization set represented larvae collected from the same season of the year and which could be used to see if similar conclusions about instar size and number could be obtained in different years.

TABLE II

Collection dates, and numbers of each larval instar per collection. Numbers in parentheses indicate number of larvae for which all 16 variables could be recorded, and which therefore could be used in Multiple Discriminant Function Analyses.

A. Standardization set of larvae, set 1

| Instar | Number collected per sampling date | | | Laboratory reared | Total |
|---|---|---|---|---|---|
| | 29-9-70 | 11-10-70 | 18-10-70 | | |
| 1 | 0 | 0 | 0 | 22 (12) | 22 (12) |
| 2 | 1 (1) | 0 | 5 ( 4) | 4 ( 2) | 10 ( 7) |
| 3 | 3 (1) | 0 | 14 ( 9) | 10 ( 9) | 27 (19) |
| 4 | 6 (3) | 0 | 12 (11) | 7 ( 5) | 25 (19) |
| 5 | 12 (3) | 0 | 17 (16) | 4 ( 4) | 33 (23) |
| 6 | 3 (2) | 0 | 13 (11) | 5 ( 5) | 21 (18) |
| 7 | 3 (2) | 0 | 19 (19) | 3 ( 3) | 25 (24) |
| 8 | 5 (5) | 0 | 13 (13) | 0 | 18 (18) |
| 9 | 7 (7) | 8 (0) | 11 ( 7) | 0 | 26 (14) |
| Total | 40 (24) | 8 (0) | 104 (90) | 55 (40) | 207 (154) |

B. Test sets of larvae

a) set 2, 3-4-71; 10 larvae per instar, total of 90 larvae.

b) set 3, 26-9-71; 5 larvae per instar, total of 45 larvae.

## Measurements and morphological features used

The following measurements and morphological features were noted for each larva. The numbers 1-16 are used throughout this account to refer to these variables. Preserved length values were recorded in mm, and all other measurements were recorded in µm. For each measurement of the three data sets, the mean, variance, standard error of the mean and coefficient of variation were calculated; these values are presented in Tables III to V.

1. Preserved length (in 90% ethanol); dorsal surface, from anterior tip of head capsule to posterior circlet of hooks.
2. Head capsule, length; dorsal surface, midline length from anterior tip of head capsule to posterior edge of head capsule. If the cervical sclerites were contiguous to the cephalic apotome, then they were included in the measurement.
3. Head capsule, width at eye spots; dorsal surface, width between the laterally placed paired eye spots. In most cases, this measurement is equivalent to the maximum head width of other authors.
4. Head capsule, postocciput width; dorsal surface (Terteryan, 1957).
5. Mandible length (AB); distance between largest apical tooth and opposing basal articulation on dorsal side of head capsule (Grenier & Feraud, 1960).
6. Number of teeth on mandible; includes number of comb teeth and inner teeth.
7. Maxillary palp length; distance from basal articulation to tip (including sensilla basiconica).
8. Antennal segment 1, length; basal segment articulating with head capsule, present from instar 2.
9. Antennal segment 2, length; present from instar 3 (first appears as an intercalated segment between segments 1 and 3).
10. Antennal segment 3, length; present in all instars.
11. Antennal segment 4, length; proximal cone-shaped segment

(morphologically a sensillum basiconicum but called a segment by simuliid taxonomists), present in all instars.

12. Number of setae in each mental row; only includes those setae anterior to mental groove.
13. Anal sclerite development; recorded as: (1) absent, (2) partially formed, weakly sclerotized, (3) present, fully sclerotized.
14. Semicircular sclerite development; recorded as: (1) absent, (2) partially formed, (3) present.
15. Imaginal buds and pupal respiratory histoblast development; recorded as: (1) absent, (2) uncertain, (3) present.
16. Semicircular sclerite articulation development; recorded as: (1) articulations not developed, (2) articulations developed.

Also noted for each larva were the number of mental teeth, and the degree of sclerotization of the maxillary palp and cephalic fans.

Later, some additional features were studied for selected larvae so as to give a fuller characterization of each instar. These were:

a) number of outer and inner cephalic rays.
b) number of rows and hooks per row to the proleg circlet of hooks.
c) number of rows and hooks per row to the posterior circlet of hooks.
d) degree of separation of the cervical sclerites from the postocciput and the cephalic apotome.

For the standardization set of larvae, live length was measured. However, as this was a difficult measurement to take objectively, it was not measured for the test sets of larvae.

All numerical analyses of these measurements were executed on an IBM 360/44 computer at the University of Canterbury Computer Centre. The computer programs used were based on programs written by Cooley & Lohnes (1962, 1971), I.B.M. (1968) and Sokal & Rohlf (1969), as well as programs written by the author. The original data are available in the author's thesis at the University of Canterbury, and in the Department of Zoology, University of Canterbury, and at the British Museum (Natural History) Library, together with print-outs of all statistics derived from them.

TABLE III

Sample sizes (N), means ($\bar{Y}$), variances ($s^2$), standard errors of means ($s_{\bar{Y}}$) and coefficients of variation (C.V.) for measurements of the standardization set of A. tillyardianum larvae

| Measurement | Instar | N | $\bar{Y}$ | $s^2$ | $s_{\bar{Y}}$ | C.V. |
|---|---|---|---|---|---|---|
| Live length | 1 | 25 | 0.40 | 0.001 | 0.004 | 4.42 |
| (mm) | 2 | 6 | 0.58 | 0.001 | 0.012 | 5.05 |
| | 3 | 13 | 0.74 | 0.015 | 0.034 | 16.68 |
| | 4 | 13 | 1.02 | 0.012 | 0.030 | 10.59 |
| | 5 | 16 | 1.38 | 0.044 | 0.052 | 15.22 |
| | 6 | 7 | 2.05 | 0.156 | 0.149 | 19.28 |
| | 7 | 6 | 2.64 | 0.303 | 0.225 | 20.82 |
| | 8 | 5 | 3.29 | 0.096 | 0.138 | 9.39 |
| | 9 | 7 | 4.75 | 0.345 | 0.222 | 12.37 |
| 1. Preserved | 1 | 18 | 0.52 | 0.006 | 0.018 | 14.49 |
| length (mm) | 2 | 9 | 0.75 | 0.011 | 0.036 | 14.24 |
| | 3 | 26 | 0.99 | 0.031 | 0.035 | 17.86 |
| | 4 | 23 | 1.32 | 0.056 | 0.051 | 17.88 |
| | 5 | 25 | 1.81 | 0.113 | 0.067 | 18.53 |
| | 6 | 21 | 2.64 | 0.155 | 0.086 | 14.96 |
| | 7 | 25 | 3.54 | 0.182 | 0.085 | 12.04 |
| | 8 | 18 | 4.21 | 0.200 | 0.105 | 10.63 |
| | 9 | 15 | 5.28 | 0.098 | 0.081 | 5.94 |
| 2. Head capsule | 1 | 17 | 112.05 | 117.999 | 2.635 | 9.69 |
| length (µm) | 2 | 10 | 153.14 | 183.767 | 4.287 | 8.85 |
| | 3 | 25 | 186.76 | 441.451 | 4.202 | 11.25 |
| | 4 | 24 | 237.45 | 592.729 | 4.970 | 10.25 |
| | 5 | 31 | 320.52 | 1 177.999 | 6.164 | 10.71 |
| | 6 | 21 | 400.37 | 1 728.220 | 9.212 | 10.54 |
| | 7 | 25 | 534.47 | 3 124.760 | 11.180 | 10.46 |
| | 8 | 18 | 613.61 | 2 334.228 | 11.388 | 7.87 |
| | 9 | 25 | 708.82 | 6 092.039 | 15.610 | 11.01 |

TABLE III -continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3. | Head capsule width at eye spots (µm) | 1 | 19 | 101.43 | 48.879 | 1.604 | 6.89 |
| | | 2 | 10 | 117.42 | 126.188 | 3.552 | 9.57 |
| | | 3 | 25 | 146.22 | 145.066 | 2.409 | 8.24 |
| | | 4 | 24 | 191.22 | 85.429 | 1.927 | 4.83 |
| | | 5 | 31 | 250.11 | 535.278 | 4.155 | 9.25 |
| | | 6 | 21 | 322.59 | 1 524.707 | 8.521 | 12.10 |
| | | 7 | 25 | 408.36 | 1 358.807 | 7.372 | 9.03 |
| | | 8 | 18 | 494.01 | 799.445 | 6.664 | 5.72 |
| | | 9 | 25 | 593.83 | 921.881 | 6.073 | 5.11 |
| 4. | Head capsule postocciput width (µm) | 1 | 18 | 93.80 | 17.821 | 0.995 | 4.50 |
| | | 2 | 10 | 102.92 | 30.346 | 1.742 | 5.35 |
| | | 3 | 25 | 129.34 | 111.488 | 2.112 | 8.16 |
| | | 4 | 24 | 191.22 | 85.430 | 1.927 | 4.83 |
| | | 5 | 31 | 219.21 | 370.367 | 3.457 | 8.78 |
| | | 6 | 21 | 272.90 | 1 438.231 | 8.276 | 13.90 |
| | | 7 | 25 | 349.03 | 1 198.199 | 6.923 | 9.92 |
| | | 8 | 18 | 427.46 | 270.978 | 3.880 | 3.85 |
| | | 9 | 25 | 511.72 | 1 555.391 | 7.888 | 7.71 |
| 5. | Mandible length (µm) | 1 | 19 | 51.47 | 1.206 | 0.252 | 2.13 |
| | | 2 | 9 | 60.08 | 17.899 | 1.410 | 7.04 |
| | | 3 | 25 | 76.16 | 29.375 | 1.084 | 7.12 |
| | | 4 | 25 | 105.35 | 49.863 | 1.412 | 6.70 |
| | | 5 | 31 | 135.33 | 144.367 | 2.158 | 8.88 |
| | | 6 | 20 | 177.65 | 219.387 | 3.312 | 8.34 |
| | | 7 | 25 | 225.38 | 262.157 | 3.238 | 7.18 |
| | | 8 | 18 | 274.90 | 171.960 | 3.091 | 4.77 |
| | | 9 | 25 | 350.15 | 224.403 | 2.996 | 4.28 |
| 7. | Maxillary palp length (µm) | 1 | 15 | 23.31 | 9.466 | 0.794 | 13.20 |
| | | 2 | 8 | 25.94 | 4.411 | 0.743 | 8.10 |
| | | 3 | 24 | 31.66 | 14.643 | 0.781 | 12.09 |
| | | 4 | 25 | 42.22 | 21.186 | 0.921 | 10.90 |
| | | 5 | 31 | 54.28 | 34.480 | 1.055 | 10.82 |
| | | 6 | 20 | 70.39 | 57.136 | 1.690 | 10.74 |
| | | 7 | 25 | 89.69 | 45.530 | 1.349 | 7.52 |
| | | 8 | 18 | 105.81 | 56.406 | 1.770 | 7.10 |
| | | 9 | 26 | 129.40 | 38.593 | 1.218 | 4.80 |

TABLE III -continued

| | | | | | | |
|---|---|---|---|---|---|---|
| 8. Antennal segment 1, length (µm) | 1 | - | - | - | - | - |
| | 2 | 9 | 22.16 | 14.860 | 1.285 | 17.40 |
| | 3 | 24 | 26.87 | 17.741 | 0.860 | 15.68 |
| | 4 | 24 | 36.47 | 14.771 | 0.785 | 10.54 |
| | 5 | 29 | 45.68 | 24.304 | 0.916 | 10.79 |
| | 6 | 21 | 60.22 | 40.555 | 1.390 | 10.57 |
| | 7 | 25 | 75.26 | 69.454 | 1.667 | 11.07 |
| | 8 | 18 | 86.14 | 16.953 | 0.971 | 4.78 |
| | 9 | 26 | 113.36 | 68.629 | 1.625 | 7.31 |
| 9. Antennal segment 2, length (µm) | 1 | - | - | - | - | - |
| | 2 | - | - | - | - | - |
| | 3 | 23 | 4.41 | 1.774 | 0.278 | 30.18 |
| | 4 | 24 | 6.93 | 1.071 | 0.211 | 14.94 |
| | 5 | 30 | 9.07 | 5.484 | 0.428 | 25.83 |
| | 6 | 21 | 11.41 | 8.017 | 0.618 | 24.83 |
| | 7 | 25 | 15.01 | 15.431 | 0.786 | 26.17 |
| | 8 | 18 | 19.07 | 16.761 | 0.965 | 21.47 |
| | 9 | 26 | 29.67 | 21.534 | 0.910 | 15.64 |
| 10. Antennal segment 3, length (µm) | 1 | 15 | 35.23 | 6.324 | 0.649 | 7.14 |
| | 2 | 7 | 38.13 | 1.919 | 0.524 | 3.63 |
| | 3 | 20 | 58.86 | 30.058 | 1.226 | 9.31 |
| | 4 | 19 | 84.06 | 78.166 | 2.028 | 10.52 |
| | 5 | 28 | 111.03 | 134.991 | 2.196 | 10.46 |
| | 6 | 20 | 144.55 | 112.537 | 2.372 | 7.34 |
| | 7 | 24 | 182.26 | 155.695 | 2.547 | 6.85 |
| | 8 | 18 | 206.68 | 73.814 | 2.025 | 8.59 |
| | 9 | 22 | 248.65 | 161.032 | 2.706 | 5.10 |
| 11. Antennal segment 4, length (µm) | 1 | 15 | 8.57 | 0.325 | 0.147 | 6.66 |
| | 2 | 7 | 8.60 | 0.653 | 0.306 | 9.40 |
| | 3 | 20 | 10.10 | 2.366 | 0.344 | 15.24 |
| | 4 | 19 | 12.23 | 1.320 | 0.264 | 9.39 |
| | 5 | 28 | 13.58 | 1.939 | 0.263 | 10.25 |
| | 6 | 20 | 15.14 | 0.949 | 0.218 | 6.43 |
| | 7 | 24 | 16.85 | 2.670 | 0.334 | 9.70 |
| | 8 | 18 | 17.92 | 1.355 | 0.274 | 6.49 |
| | 9 | 22 | 19.28 | 1.691 | 0.277 | 6.75 |

TABLE IV

Means ($\bar{Y}$), variances ($s^2$), standard errors of means ($s_{\bar{Y}}$) and coefficients of variation (C.V.) for measurements of the test set 3-4-71 A. tillyardianum larvae (N = 10)

| Measurement | Instar | $\bar{Y}$ | $s^2$ | $s_{\bar{Y}}$ | C.V. |
|---|---|---|---|---|---|
| 1. Preserved length (mm) | 1 | 0.61 | 0.003 | 0.016 | 8.40 |
| | 2 | 0.82 | 0.004 | 0.020 | 7.59 |
| | 3 | 1.11 | 0.008 | 0.029 | 8.25 |
| | 4 | 1.60 | 0.018 | 0.042 | 8.29 |
| | 5 | 1.93 | 0.025 | 0.050 | 8.28 |
| | 6 | 2.41 | 0.083 | 0.091 | 11.94 |
| | 7 | 3.15 | 0.025 | 0.050 | 5.03 |
| | 8 | 4.09 | 0.041 | 0.064 | 4.95 |
| | 9 | 5.04 | 0.115 | 0.107 | 6.71 |
| 2. Head capsule length (µm) | 1 | 116.23 | 51.272 | 2.264 | 6.16 |
| | 2 | 150.63 | 73.160 | 2.705 | 5.68 |
| | 3 | 214.31 | 133.445 | 3.653 | 5.39 |
| | 4 | 271.50 | 594.176 | 7.708 | 8.98 |
| | 5 | 335.61 | 668.444 | 8.176 | 7.70 |
| | 6 | 416.10 | 627.638 | 7.922 | 6.02 |
| | 7 | 522.68 | 464.214 | 6.833 | 4.12 |
| | 8 | 641.70 | 124.297 | 3.526 | 1.74 |
| | 9 | 728.66 | 499.565 | 7.068 | 3.07 |
| 3. Head capsule width at eye spots (µm) | 1 | 100.49 | 14.741 | 1.215 | 3.82 |
| | 2 | 123.76 | 56.265 | 2.372 | 6.06 |
| | 3 | 158.93 | 68.653 | 2.620 | 5.21 |
| | 4 | 202.36 | 187.638 | 4.332 | 6.77 |
| | 5 | 259.91 | 150.756 | 3.883 | 4.72 |
| | 6 | 292.36 | 538.781 | 7.340 | 7.94 |
| | 7 | 369.38 | 997.111 | 9.986 | 8.55 |
| | 8 | 472.32 | 497.690 | 7.055 | 4.72 |
| | 9 | 560.64 | 388.425 | 6.232 | 3.52 |

TABLE IV -continued

| | | | | | |
|---|---|---|---|---|---|
| 4. Head capsule, postocciput width (µm) | 1 | 94.94 | 7.335 | 0.856 | 2.85 |
| | 2 | 108.98 | 69.015 | 2.627 | 7.62 |
| | 3 | 139.77 | 140.071 | 3.743 | 8.47 |
| | 4 | 178.07 | 204.407 | 4.521 | 8.03 |
| | 5 | 229.52 | 230.808 | 4.804 | 6.62 |
| | 6 | 248.23 | 447.116 | 6.687 | 8.52 |
| | 7 | 313.74 | 720.256 | 8.487 | 8.55 |
| | 8 | 392.74 | 258.159 | 5.081 | 4.09 |
| | 9 | 473.78 | 644.502 | 8.028 | 5.36 |
| 5. Mandible length (µm) | 1 | 50.69 | 4.919 | 0.701 | 4.38 |
| | 2 | 61.05 | 2.872 | 0.536 | 2.78 |
| | 3 | 81.77 | 9.565 | 0.978 | 3.78 |
| | 4 | 106.26 | 81.885 | 2.862 | 8.52 |
| | 5 | 132.83 | 25.131 | 1.585 | 3.77 |
| | 6 | 167.28 | 199.188 | 4.463 | 8.44 |
| | 7 | 202.00 | 157.829 | 3.973 | 6.22 |
| | 8 | 254.49 | 101.963 | 3.193 | 3.97 |
| | 9 | 316.65 | 102.056 | 3.195 | 3.19 |
| 7. Maxillary palp length (µm) | 1 | 22.16 | 4.976 | 0.705 | 10.07 |
| | 2 | 28.51 | 3.714 | 0.610 | 6.76 |
| | 3 | 33.84 | 12.596 | 1.122 | 10.49 |
| | 4 | 44.79 | 9.397 | 0.969 | 6.84 |
| | 5 | 53.97 | 8.609 | 0.928 | 5.44 |
| | 6 | 63.49 | 31.905 | 1.786 | 8.90 |
| | 7 | 81.06 | 27.700 | 1.664 | 6.49 |
| | 8 | 98.34 | 57.507 | 2.398 | 7.71 |
| | 9 | 120.53 | 32.422 | 1.801 | 4.72 |
| 8. Antennal segment 1 length (µm) | 1 | - | - | - | - |
| | 2 | 22.74 | 1.296 | 0.360 | 5.00 |
| | 3 | 26.36 | 13.605 | 1.166 | 13.99 |
| | 4 | 35.56 | 10.620 | 1.031 | 9.16 |
| | 5 | 44.93 | 7.229 | 0.850 | 5.98 |
| | 6 | 56.29 | 43.150 | 2.077 | 11.67 |
| | 7 | 63.50 | 9.393 | 0.969 | 4.83 |
| | 8 | 79.63 | 29.062 | 1.705 | 6.77 |
| | 9 | 105.11 | 59.681 | 2.443 | 7.35 |

TABLE IV -continued

| | | | | | | |
|---|---|---|---|---|---|---|
| 9. | Antennal segment 2 length (µm) | 1 | - | - | - | - |
| | | 2 | - | - | - | - |
| | | 3 | 4.47 | 1.153 | 0.340 | 24.03 |
| | | 4 | 5.92 | 1.137 | 0.337 | 18.01 |
| | | 5 | 7.49 | 1.712 | 0.414 | 17.47 |
| | | 6 | 8.93 | 1.782 | 0.422 | 14.95 |
| | | 7 | 11.73 | 3.358 | 0.580 | 15.62 |
| | | 8 | 14.98 | 6.571 | 0.811 | 17.11 |
| | | 9 | 22.06 | 27.898 | 1.670 | 23.94 |
| 10. | Antennal segment 3 length (µm) | 1 | 32.26 | 3.358 | 0.580 | 5.68 |
| | | 2 | 35.76 | 4.344 | 0.659 | 5.83 |
| | | 3 | 57.61 | 31.630 | 1.779 | 9.76 |
| | | 4 | 83.66 | 12.485 | 1.117 | 4.22 |
| | | 5 | 108.43 | 23.585 | 1.536 | 4.48 |
| | | 6 | 130.87 | 150.578 | 3.880 | 9.38 |
| | | 7 | 160.42 | 76.342 | 2.763 | 5.45 |
| | | 8 | 197.52 | 85.751 | 2.928 | 4.69 |
| | | 9 | 236.85 | 103.632 | 3.219 | 4.30 |
| 11. | Antennal segment 4 length (µm) | 1 | 8.47 | 0.667 | 0.258 | 9.64 |
| | | 2 | 8.94 | 0.389 | 0.197 | 6.98 |
| | | 3 | 10.80 | 0.544 | 0.233 | 6.83 |
| | | 4 | 11.81 | 1.763 | 0.420 | 11.24 |
| | | 5 | 12.98 | 0.935 | 0.306 | 7.45 |
| | | 6 | 13.78 | 1.331 | 0.365 | 8.37 |
| | | 7 | 14.83 | 1.382 | 0.372 | 7.93 |
| | | 8 | 16.90 | 2.498 | 0.500 | 9.35 |
| | | 9 | 18.46 | 3.685 | 0.607 | 10.40 |

TABLE V

Means ($\bar{Y}$), variances ($s^2$), standard errors of means ($s_{\bar{Y}}$) and coefficients of variation (C.V.) for measurements of the test set 26-9-71 A. tillyardianum larvae (N = 5)

| Measurement | Instar | $\bar{Y}$ | $s^2$ | $s_{\bar{Y}}$ | C.V. |
|---|---|---|---|---|---|
| 1. Preserved length (mm) | 1 | 0.64 | 0.003 | 0.022 | 7.86 |
| | 2 | 0.93 | 0.003 | 0.024 | 5.84 |
| | 3 | 1.11 | 0.004 | 0.029 | 5.87 |
| | 4 | 1.51 | 0.010 | 0.044 | 6.58 |
| | 5 | 2.09 | 0.042 | 0.092 | 9.87 |
| | 6 | 2.61 | 0.008 | 0.039 | 3.33 |
| | 7 | 3.50 | 0.061 | 0.110 | 7.02 |
| | 8 | 4.39 | 0.311 | 0.250 | 12.72 |
| | 9 | 5.65 | 0.116 | 0.153 | 6.04 |
| 2. Head capsule length (µm) | 1 | 123.28 | 44.247 | 2.975 | 5.40 |
| | 2 | 156.38 | 104.387 | 4.569 | 6.53 |
| | 3 | 212.12 | 199.767 | 6.321 | 6.66 |
| | 4 | 283.08 | 100.766 | 4.489 | 3.55 |
| | 5 | 355.48 | 97.252 | 4.410 | 2.77 |
| | 6 | 448.24 | 309.668 | 7.870 | 3.93 |
| | 7 | 540.80 | 2 045.533 | 20.226 | 8.36 |
| | 8 | 670.52 | 633.363 | 11.257 | 3.75 |
| | 9 | 760.36 | 326.465 | 8.080 | 2.38 |
| 3. Head capsule width at eye spots (µm) | 1 | 99.74 | 21.623 | 2.080 | 4.66 |
| | 2 | 126.20 | 44.880 | 2.996 | 5.31 |
| | 3 | 165.83 | 55.227 | 3.324 | 4.48 |
| | 4 | 215.04 | 115.493 | 4.806 | 5.00 |
| | 5 | 263.54 | 389.172 | 8.822 | 7.49 |
| | 6 | 319.68 | 180.592 | 6.010 | 4.20 |
| | 7 | 392.36 | 336.665 | 8.206 | 4.68 |
| | 8 | 506.88 | 254.233 | 7.131 | 3.15 |
| | 9 | 597.56 | 74.028 | 3.848 | 1.44 |

TABLE V -continued

| | | | | | |
|---|---|---|---|---|---|
| 4. Head capsule, postocciput width (µm) | 1 | 95.12 | 34.847 | 2.640 | 6.20 |
| | 2 | 114.58 | 33.882 | 2.603 | 5.08 |
| | 3 | 152.04 | 156.783 | 5.600 | 8.24 |
| | 4 | 193.32 | 214.392 | 6.548 | 7.57 |
| | 5 | 233.86 | 253.198 | 7.116 | 6.80 |
| | 6 | 278.20 | 331.778 | 8.146 | 6.55 |
| | 7 | 332.88 | 362.372 | 8.513 | 5.72 |
| | 8 | 442.16 | 377.066 | 8.684 | 4.39 |
| | 9 | 511.00 | 213.159 | 6.529 | 2.86 |
| 5. Mandible length (µm) | 1 | 51.26 | 5.873 | 1.084 | 4.73 |
| | 2 | 63.36 | 8.123 | 1.275 | 4.50 |
| | 3 | 83.82 | 18.997 | 1.949 | 5.20 |
| | 4 | 106.00 | 60.780 | 3.487 | 7.35 |
| | 5 | 133.18 | 66.432 | 3.645 | 6.12 |
| | 6 | 170.30 | 174.150 | 5.902 | 7.75 |
| | 7 | 212.16 | 167.693 | 5.791 | 6.10 |
| | 8 | 255.58 | 291.786 | 7.639 | 6.68 |
| | 9 | 327.64 | 253.328 | 7.118 | 4.86 |
| 7. Maxillary palp length (µm) | 1 | 18.18 | 23.127 | 2.151 | 26.45 |
| | 2 | 25.54 | 4.553 | 0.954 | 8.35 |
| | 3 | 33.44 | 2.523 | 0.714 | 4.75 |
| | 4 | 45.20 | 8.940 | 1.337 | 6.62 |
| | 5 | 55.00 | 13.065 | 1.617 | 6.57 |
| | 6 | 69.44 | 26.208 | 2.290 | 7.37 |
| | 7 | 82.38 | 35.557 | 2.667 | 7.24 |
| | 8 | 103.98 | 15.872 | 1.782 | 3.83 |
| | 9 | 129.00 | 70.025 | 3.742 | 6.65 |
| 8. Antennal segment 1 length (µm) | 1 | - | - | - | - |
| | 2 | 22.76 | 3.503 | 0.837 | 8.22 |
| | 3 | 25.54 | 4.553 | 0.954 | 8.35 |
| | 4 | 36.56 | 6.823 | 1.168 | 7.14 |
| | 5 | 47.80 | 2.480 | 0.704 | 3.29 |
| | 6 | 57.58 | 3.082 | 0.785 | 3.05 |
| | 7 | 67.08 | 38.892 | 2.789 | 9.30 |
| | 8 | 80.92 | 5.692 | 1.067 | 2.95 |
| | 9 | 106.56 | 11.348 | 1.507 | 3.16 |

TABLE V -continued

| | | | | | | |
|---|---|---|---|---|---|---|
| 9. | Antennal | 1 | - | - | - | - |
| | segment 2 | 2 | - | - | - | - |
| | length (µm) | 3 | 5.68 | 0.152 | 0.174 | 6.86 |
| | | 4 | 7.20 | 0.980 | 0.443 | 13.75 |
| | | 5 | 8.64 | 2.103 | 0.649 | 16.78 |
| | | 6 | 10.06 | 4.278 | 0.925 | 20.56 |
| | | 7 | 14.12 | 5.577 | 1.056 | 16.72 |
| | | 8 | 19.24 | 29.123 | 2.413 | 28.05 |
| | | 9 | 26.68 | 13.127 | 1.620 | 13.58 |
| 10. | Antennal | 1 | 33.46 | 3.298 | 0.812 | 5.43 |
| | segment 3 | 2 | 37.64 | 1.583 | 0.563 | 3.34 |
| | length (µm) | 3 | 59.60 | 5.715 | 1.069 | 4.01 |
| | | 4 | 84.94 | 19.758 | 1.988 | 5.23 |
| | | 5 | 103.82 | 7.882 | 1.256 | 2.70 |
| | | 6 | 130.38 | 27.832 | 2.359 | 4.05 |
| | | 7 | 154.96 | 73.883 | 3.844 | 5.55 |
| | | 8 | 192.60 | 22.430 | 2.118 | 2.48 |
| | | 9 | 248.36 | 193.612 | 6.223 | 5.60 |
| 11. | Antennal | 1 | 8.60 | 0.000 | 0.000 | 0.00 |
| | segment 4 | 2 | 8.62 | 1.052 | 0.459 | 11.90 |
| | length (µm) | 3 | 9.80 | 0.450 | 0.300 | 6.85 |
| | | 4 | 12.30 | 0.575 | 0.339 | 6.16 |
| | | 5 | 13.76 | 0.508 | 0.319 | 5.18 |
| | | 6 | 15.52 | 0.392 | 0.280 | 4.04 |
| | | 7 | 16.68 | 1.697 | 0.583 | 7.81 |
| | | 8 | 18.44 | 1.458 | 0.540 | 6.55 |
| | | 9 | 19.00 | 0.450 | 0.300 | 3.53 |

## Measuring methods and their effects on statistical tests

### Measuring methods

Measurements were taken with a 100 unit eyepiece graticule, and were accurate to the nearest 1/2 unit (7.3 µm at x100, 1.8 µm at x400, 1.1 µm at x600 and 0.7 µm at x1000). Since measurements were taken using the highest possible magnification for the whole structure to remain in the field of view, different magnifications were used for different instars to measure the same structure. This multiple magnification approach allowed a more accurate separation of early instars than would have been possible using the one magnification approach characteristic of earlier studies.

To measure preserved length, larvae were mounted dorsal surface up on a microscope slide in a thin layer of petroleum jelly. The length of instars 1 to 3 was measured with a compound microscope, instars 4 to 9 with a dissecting microscope. Morphological features of the thorax and abdomen of all instars were checked at the same time.

Head capsule measurements of instars 1 to 3 were also taken at this point. For older instars, however, before taking the measurements with a compound microscope the larvae were decapitated and the head capsules were remounted on a microscope slide.

To obtain the remaining measurements of instars 1 to 3, whole larvae were mounted in glycerine on a microscope slide and gently squashed until the required structures were clearly visible. Head capsules of instars 4 to 9 were cut laterally in an anterior-posterior plane, and the

dorsal and ventral halves were mounted in glycerine and splayed before these measurements were taken. The thorax and abdomen of selected larvae of older instars were mounted in glycerine on a microscope slide so that additional morphological features used to characterize the instars could be noted (Crosby, in press).

## Sampled randomization tests

For any measurement of an instar there are only a limited number of values that a measurement can take. This is a consequence of measuring on a scale of "eyepiece graticule units" having fewer divisions than the μm scale to which the "units" were converted. In many cases, the values of measurements of an instar did not appear to be normally distributed, either being skewed, or evenly spread over the instar. The statistical tests used here depended upon data being normally distributed, as did the probability tables which indicated the significance of the tests. Although the tests for skewness, kurtosis, and the Kolmogorov-Smirnov $D_{max}$ statistic (Sokal & Rohlf, 1969) for these measurements indicated that there were no obvious departures from normality, I thought that the small sample sizes might have prevented any non-normality from being demonstrated.

Therefore, a series of sampled randomization tests using a Monte Carlo approach was made to test whether the tabled probability values for a statistic corresponded to the "probability" obtained for a statistic by chance alone for a sample (Sokal & Rohlf, 1969). Tested were the t-test probabilities of means and the F-ratio probabilities of variances.

Three different data sets were used for these comparisons:

1. Instar 2, mandible length; comparison between standardization set ($\underline{n}$ = 9) and test set 3-4-71 ($\underline{n}$ = 10); no significant difference between means of mandible lengths, highly significant difference between variances.
2. Instar 8, head capsule length; comparison between standardization set ($\underline{n}$ = 18) and test set 3-4-71 ($\underline{n}$ = 10); significant difference between means, highly significant difference between variances.
3. Instars 7 and 8, antennal segment 3 length; comparison between individuals assigned to these instars of standardization set (instar 7, $\underline{n}$ = 24; instar 8, $\underline{n}$ = 18); highly significant difference between means, no significant difference between variances.

For each randomization test run, data were arranged into 1 000 random partitions using the original sample sizes and the appropriate statistic was calculated for every partition. Any statistic of a partition more deviant than (i.e. "worse than") the statistic observed for the sample was counted. The total number of these random partition statistic deviants was divided by 10 to give the percentage of "worse than" cases in that randomization test run. Confidence limits for this percentage were given by Table W of Rohlf & Sokal (1969). Finally, the percentage "worse than" cases for the randomization test statistic was compared with the tabled probability (percentage) "worse than" cases expected according to the observed sample statistic (Tables VI and VII).

Effect of measuring scale on t-test statistic

There was a close correspondence between tabled levels of significance of the observed values and of those levels of significance

TABLE VI

Results of sampled randomization tests for t-test probabilities of means compared with tabled probabilities of observed t values

| Data set | Observed value of t | t-table probability (%) | Randomization probability (%) | Confidence limits for probability (%) |
|---|---|---|---|---|
| 1 | 0.675 | > 50 | 67 | 64-70 |
| 2 | 2.076 | 2.5-5.0 | 2.9 | 2.1-4.2 |
| 3 | 7.126 | < 0.1 | 0 | 0.0-0.4 |

TABLE VII

Results of sampled randomization tests for F-ratio probabilities of variances compared with tabled probabilities of observed F-ratio values

| Data set | Observed value of F-ratio | F-table probability (%) | Randomization probability (%) | Confidence limits for probability (%) |
|---|---|---|---|---|
| 1 | 6.303 | 0.5-1.0 | 52 | 49-55 |
| | | | 51 | 48-54 |
| 2 | 12.407 | < 0.1 | 37 | 34-40 |
| | | | 38 | 35-41 |
| 3 | 2.109 | 5-10 | 57 | 54-60 |

derived from the randomization test values. For example, in data set 2 the observed t value indicated that only 2.5 to 5.0% of all other samples would have a greater difference between means by chance alone (be "worse than"); that is, the means of the two samples were significantly different (Table VI ). The result of the randomized partitioning of the data set was that 2.1 to 4.2% of the partitioned t values were greater than ("worse than") the observed t value. Therefore the conclusion derived from the observed t value -- that there was a significant difference between means -- is justified.

It appears then as if the performance of the t-test has not been affected by the variables being measured on an "eyepiece graticule scale". Any significant differences between means of samples can be expected to be true differences; conversely, the absence of any significant differences between means of samples can be interpreted as showing the samples came from a homogeneous population.

Effect of measuring scale on F-ratio statistic

There was no correspondence between tabled levels of significance of the observed values and those levels of significance derived from the randomization test values. For all examples there were many more "worse than" cases in a randomization test run than predicted by an observed F-ratio. In data sets 1 and 2, the observed F-ratios indicated that there were highly significant differences between variances of the samples: the randomization test runs strongly suggested that there were no such differences. The striking lack of correspondence was further demonstrated by replicate randomization runs giving the same results (Table VII). It must be concluded that measuring

variables on an "eyepiece graticule scale" affected variances of samples to such an extent that derived F-ratios can not be meaningfully interpreted according to levels of significance given by F-tables.

Student's t-test or adjusted t-test to test means

Student's t-test relies on the variances of the two samples tested being homogeneous; in cases where variances are hetereogeneous, an adjusted t-test may be used (Sokal & Rohlf, 1969). Although the appropriate t-test for each case has been used throughout this study, according to the results of the randomization tests it is possible that this distinction need not have been made.

Observed t-values for data sets 1 and 2 were those calculated by an adjusted t-test. Yet results of the randomization tests, by using Student's t-test and ignoring hetereogeneous variances, were the same in both cases (Table VI ). This could be regarded as further evidence for not needing to use an adjusted t-test for my instar determination data. However, it could be that this apparent robustness of the t-test to hetereogeneous variances was mainly due to similar-sized samples being tested (Bradley, 1968).

## Division into instar groupings

Once all the larvae of the standardization set had been measured, they were placed into provisional instar groupings based upon morphological features and general size. The morphological features initially used to separate the groupings were:

Instar group 1, egg burster present; antenna 2-segmented; anal sclerite absent.

2, antenna 3-segmented; anal sclerite present.

3, antenna 4-segmented.

4, semicircular sclerite present; backwardly directed strut from each anterior arm of anal sclerite present.

5, terminal expansions on semicircular sclerite.

9, imaginal buds and pupal respiratory histoblast containing recognizable structures; cervical sclerites separated from postocciput.

In several cases, morphological features of the next instar group could also be seen (e.g. number of setae on mentum), and this assisted in deciding the limits of an instar group.

Instar groupings 6, 7 and 8 were mainly separated on the basis of general size, and of the relative size of the imaginal buds and pupal respiratory histoblast. Morphological evidence confirming the validity of these instar groups was obtained at a later date after a closer examination of the larvae.

The measurements of these provisional instar groupings were inspected to see if they formed relatively discrete groups. Larvae which had measurements intermediate between two instar groupings, or for which the measurements and morphology did not correspond, were re-examined. The measurements were then analyzed to see if the instar groups could be justified statistically.

## Methods of analyzing instars

Four techniques were used to analyze the measurements of the instar groups:

1. Frequency distributions of measurements.
2. Brooks' rule (Dyar's rule).
3. Student's t-test.
4. Multiple discriminant function analysis.

If these techniques indicated that instar groups constructed on the basis of measurements corresponded to those formed by morphological differences, then I considered the instar groupings were justified.

## Separating instars by using frequency distributions of measurements

Plotting the frequency distributions of measured structures has been used as a method for separating instars of simuliids by several authors (Grenier & Feraud, 1960; Smith, unpublished 1969; Johnson & Pengelly, 1970; Jedlička, 1972). In these cases the defined cut-off points between some instars appear to be suspect.

A frequency distribution of mandible length for A. tillyardianum, using the standardization set of data, is given in fig. 1. This measurement was considered to provide a good indication of the instar of an individual, as indicated by morphological characters. However, it can be seen that it is not possible to group the early instars by clear-cut divisions in the frequency distribution, and in fact the frequency distribution resembles that presented by Grenier & Feraud (1960) for mandible length of Simulium (Edwardsellum) damnosum in which the divisions were also indistinct. Another measurement used in an attempt to distinguish the instar of A. tillyardianum individuals, length of antennal segment 3, is also graphed in fig. 1; the boundaries between instars are likewise indistinct.

Jedlička (1972) found that for Simulium (Odagmia) ornatum two measurements (head breadth and width of cephalic apotome) plotted one against the other provided the best separation of larvae into seven instar groups. When mandible length is plotted against antennal segment 3 length in a similar manner for A. tillyardianum, separation of instars is better than taking each measurement singly (fig. 1(b)). However, the boundaries between instars are less distinct than the visual

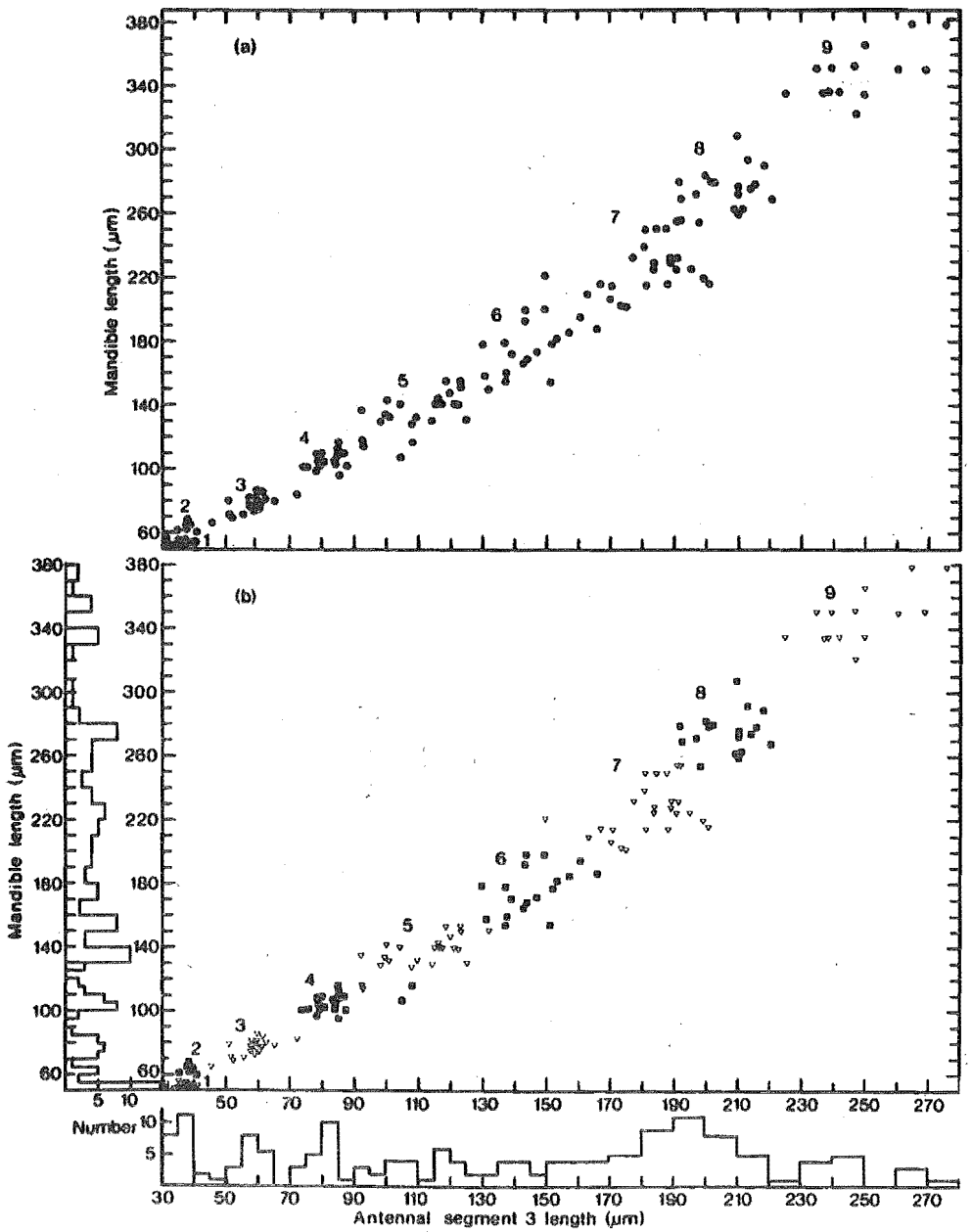



FIG. 1. Frequency histograms of mandible length and antennal segment 3 length for the standardization set of *A. tillyardianum* larvae; and scattergrams of these measurements, (a) plotted with a uniform symbol, and (b) plotted with different symbols to separate adjacent instars.

impression suggested by fig. 1(b), as is shown when all instars are plotted with a uniform symbol (fig. 1(a)). The boundaries still remained doubtful when measurements were converted to a logarithmic scale that should have enhanced separation of early instars.

Although the separation of the instars of A. tillyardianum by using the above two measurements of the standardization set of data might be considered acceptable, if the measurements of individuals from the test sets of data are also plotted, the boundaries between instars become very indistinct. Separation of instars by frequency distributions of measurements is likewise indecisive when data of the test sets are included.

## Brooks' Rule (Dyar's Rule)

Brooks' rule states that with each moult of an instar, there is usually a geometric increase in the size of sclerotized structures. Until recently, this relationship was known as Dyar's rule, but as it had actually been documented by Brooks (1886) four years before Dyar's (1890) paper, the latter name should take priority (Crosby, 1973). When the logarithm of measurement is plotted against the appropriate instar number, a straight line should be obtained. If there is any marked deviation from a straight line, it may indicate that either an instar has been missed, or two or more instars have been lumped, at the point of the deviation. It is assumed that the structure measured has a similar growth pattern from instar to instar, since changing growth patterns of a structure may result in erroneous conclusions being made. A further assumption is that all instars live for about the same period of time (Richards, 1949): this is the case in A. tillyardianum (Crosby, in prep.). However, it has been shown recently that for two species of Ectobius (Dictyoptera: Blattidae) this criterion does not greatly influence the accuracy of the logarithmic progression (Brown & Davies, 1972).

### Application of Brooks' rule to A. tillyardianum measurements

Except for antennal segment 3 length, the measurements followed a logarithmic progression, indicating that there were no missing instars in the series (fig. 2). Although there is not a perfect straight line relationship for any one measurement, when all are considered in combination the relationship clearly holds. It can be concluded, therefore, that instar groupings formed by morphological characters and by measurements are the same.

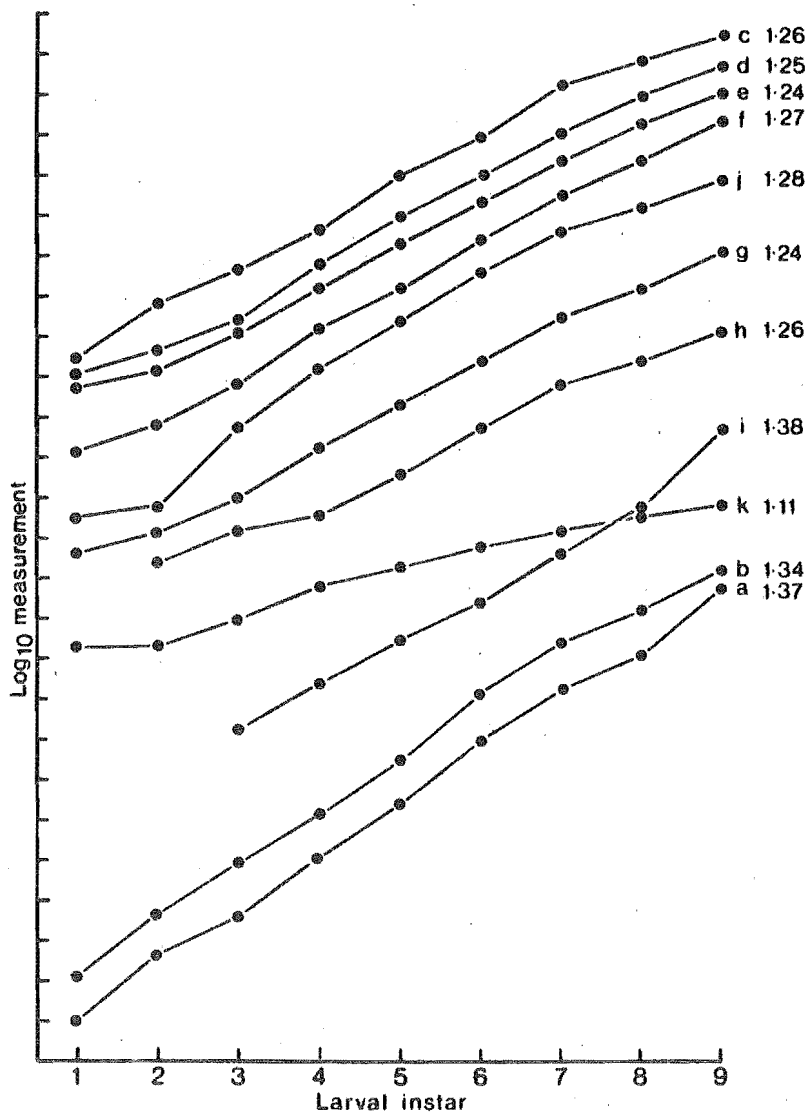



FIG. 2. Logarithms of measurements plotted against larval instar for the standardization set of *A. tillyardianum* larvae, with average growth ratios for the measurements. (a,b) body lengths, (a) live length, (b) preserved length; (c-e) head capsule measurements, (c) length, (d) width at eye spots, (e) postocciput width; (f,g) mouthparts, (f) mandible length, (g) maxillary palp length; (h-k) length of antennal segments, (h) segment 1, (i) segment 2, (j) segment 3, (k) segment 4.

Brown & Davies (1972) developed a criterion to test measurements for logarithmic progression. They calculated the ratio of the highest to lowest growth-ratio, and stated that "A structure exhibiting completely regular growth throughout all its postembryonic stages would therefore have a final ratio of 1.0, while those with final ratios of 1.0-1.05 would agree closely with Dyar's Law and those with values of 1.05-1.10 would show reasonable approximation to it." Calculation of these ratios for A. tillyardianum showed that according to this criterion none of the measurements conformed to Brooks' rule. This result appears to be caused mainly by differences in growth ratios in the first two or three instars and the final instar. Probably the different ratios for the first few instars are a result of using different magnifications for measuring these instars than were used for later instars. The ratios for measurements of the final instar are smaller than would be expected in every case; this maybe because for most of its duration the final instar is a pharate pupa (Hinton, 1958).

When these factors are taken into account by considering the 95% confidence intervals of the means and the means of other collections, the calculated ratios for most structures do show a "reasonable approximation" to Brooks' rule according to the criterion proposed by Brown & Davies (1972).

Patterns of growth

Different growth ratios for different structures are apparent in fig. 2. The ratios for body length are about 1.35, head measurements and mouthparts have ratios of about 1.26, whereas ratios for antennal segment lengths are variable.

The head width ratio (1.25) is comparable to that found by Johnson & Pengelly (1970) for Simulium (Simulium) rugglesi and by Smith (unpublished 1969) for Prosimulium (Prosimulium) hirtipes. Mansingh, Steele & Helson (1972) found that the difference in average head width between any two successive categories (= tentative instars) of Prosimulium (Prosimulium) mysticum gave a growth ratio of about 1.38. Although this is a higher ratio than the others, it may be explained by the larger size ultimately attained by the species.

In A. tillyardianum, the differences in ratios between body length and head measurements result in the head capsule becoming relatively smaller in relation to the overall size of the larvae from instar to instar.

The increase in length of antennal segment 3 with instar does not exhibit a convincing logarithmic relationship; it is in fact better described by a second degree polynomial. There appear to be three different phases of growth for antennal segment 3: (1) little or no increase between instars 1 and 2, (2) a logarithmic increase between instars 2 and 5, and (3) a constant length increment between instars 5 and 9. Brooks' rule can not apply to such a growth pattern.

The smallest growth ratio for any structure was 1.11 for antennal segment 4 length, a structure that is about 8 µm long in instar 1, and by instar 9 is still only about 20 µm long. Because such small differences between instars are found it is an unreliable measurement to make for determining if all instars are present in a measured series.

Detection of missing instars

Until now, it has been assumed that deviations from a logarithmic progression are seen quite easily. However, few measurements have a definite straight line logarithmic progression, in most instances there are some deviations around the straight line relationship. This poses the problem; how large a deviation can be considered a significant deviation from a straight line showing that the instar categories have been incorrectly formed?

To answer the above question, known middle instars of A. tillyardianum were lumped to give modified instar groupings. The groupings were analyzed first by growth ratios and then by graphing to see if any significant deviations could be detected in the logarithmic progressions. Significant deviations would signify that instars had been lumped or missed, or that different generations had been mixed in the measured series. If an individual had been measured from instar to instar, a significant deviation could denote that abnormal growth had occurred.

Growth ratios -- It was possible to show that instars of A. tillyardianum had been lumped together using growth ratios. It was found that when growth ratios of two successive instar groupings differed by more than about 12-15% lumping was indicated, and when ratios differed by more than about 20% it was evidence that several instars had been lumped. Table VIII, using the example of mandible length, demonstrates these relationships.

My results together with published accounts allowed a "growth ratio rule" to be formulated for checking the significance of a deviation around a logarithmic progression.

TABLE VIII

Application of "growth ratio rule" to mandible length of A. tillyardianum, standardization set of data. Underlining a percentage difference between ratios indicates that it is a significant deviation from a straight line logarithmic progression; * = lumped instar categories

| Instar | 9 instars | | | Case 1, 8 "instars" | | | Case 2, 7 "instars" | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean length (µm) | Growth ratio | Difference between ratios (%) | Mean length (µm) | Growth ratio | Difference between ratios (%) | Mean length (µm) | Growth ratio | Difference between ratios (%) |
| 1 | 51.5 | | | 51.5 | | | 51.5 | | |
| | | 1.17 | | | 1.17 | | | 1.17 | |
| 2 | 60.1 | | 8.5 | 60.1 | | 8.5 | 60.1 | | 8.5 |
| | | 1.27 | | | 1.27 | | | 1.27 | |
| 3 | 76.2 | | 8.7 | 76.2 | | 8.7 | 76.2 | | |
| | | 1.38 | | | 1.38 | | | 1.59 | <u>25.2</u> |
| 4 | 105.3 | | -7.8 | 105.3 | | -7.8 | | | |
| | | 1.28 | | | 1.28 | | *121.4 | | |
| 5 | 135.3 | | 2.3 | 135.3 | | <u>14.1</u> | | | 2.5 |
| | | 1.31 | | | 1.46 | | | 1.63 | |
| 6 | 177.6 | | -3.2 | | | | | | |
| | | 1.27 | | *197.4 | | -5.0 | *197.4 | | <u>-17.3</u> |
| 7 | 225.4 | | -4.1 | | 1.39 | | | 1.39 | |
| | | 1.22 | | | | -9.4 | | | -9.4 |
| 8 | 274.9 | | 4.1 | 279.4 | | | 279.4 | | |
| | | 1.27 | | | 1.27 | | | 1.27 | |
| 9 | 350.1 | | | 350.1 | | | 350.1 | | |

a) If two successive ratios differ more than about 10-15%, the deviation is probably significant; and

b) if two successive ratios differ more than about 20%, it is almost certain that the deviation is significant (i.e. instars have been lumped or missed).

For example, Brooks (1886) considered that a stage was missing in the erichthi larvae series of Lysiosquilla minuta Brooks (Crustacea: Squillidae) he measured, and the "growth ratio rule" indicates that there is a 29% difference between ratios for his specimens 3 and 4. The four zoeal stages of Ozius truncatus H. Milne Edwards (Crustacea: Xanthidae) reared by Wear (1968) conform to the "growth ratio rule" as there are no significant differences between the ratios. Dyar's (1890) conclusions based on measurements of Lepidoptera larvae hold when the "growth ratio rule" is applied, except in a few cases when the larvae appeared to be too small to be accurately measured. The growth ratios given by Savage (1971) for Sigara concinna (Fieber) (Hemiptera: Corixidae) show that no stages were missed, as is also the case for Xanthocnemis zealandica McLachlan (Odonata : Coenagrionidae) larvae measured by Scott (1971). The calculated percentages for Metriocnemus martinii Thienemann (Diptera : Chironomidae) given by Kitching (1970) are borderline, but the larval head capsule widths of the different instars do not overlap indicating that the groups have been correctly formed. Similarly, the Trichoptera larvae measured by Michaelis (pers. comm.) (Polyplectropus puerilis (McLachlan), family Polycentropodidae; Psilochorema tautoru McFarlane, family Rhyacophilidae) give borderline percentages possibly because of the small size of the early instars.

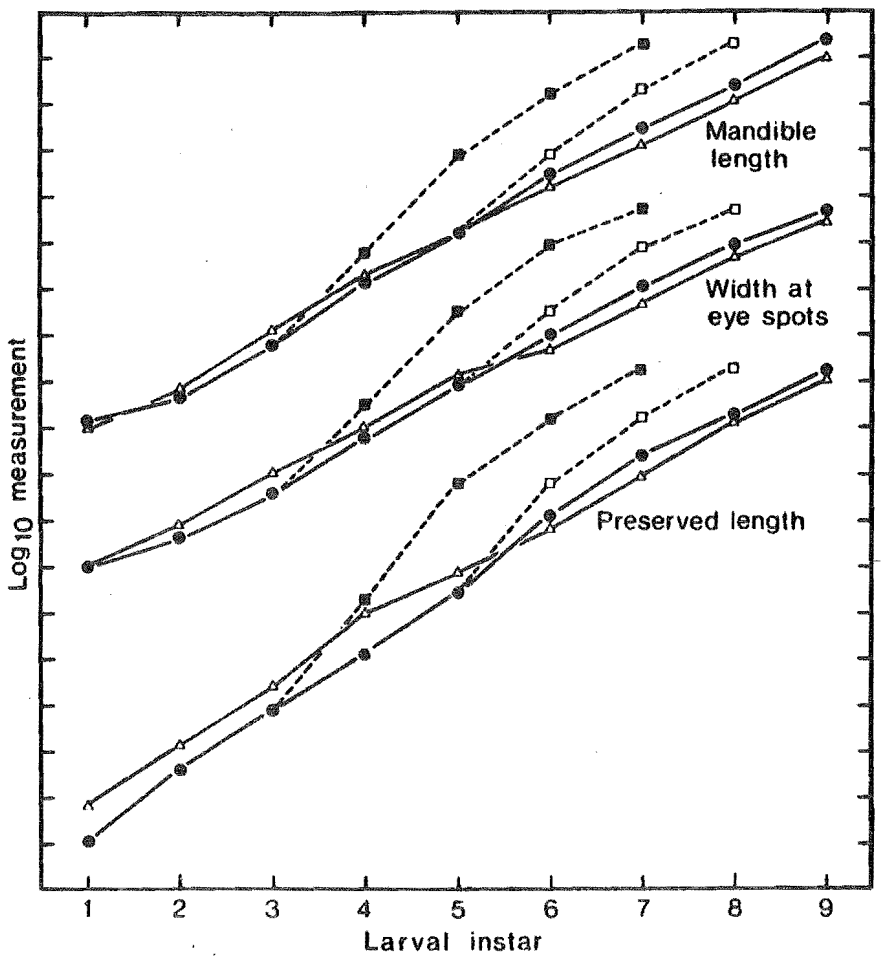



FIG. 3. Effect of different sampling times and lumping instars on the logarithmic progressions of three measurements of *A. tillyardianum* larvae. ●, standardization data; △, 3-4-71 test set of data; □, instars 6 and 7 lumped to give an "eight" instars progression; ■, instars 4 and 5, 6 and 7 lumped to give a "seven" instars progression.

In the case of Lithocolletis blancardella Fabricius (Lepidoptera : Gracillariidae), a significant deviation between larval instars 3 and 4 is caused by a change in morphology of the head capsule (Pottinger & LeRoux, 1971). This example emphasizes that significant deviations shown by the "growth ratio rule" may be caused by a change in growth rate of a structure between larval instars.

Graphing logarithmic progressions -- Because decisions about the significance of deviations around logarithmic progressions are subjective when graphing, it is not possible to define when a deviation should begin to be regarded with suspicion. This is exemplified by the results of lumping instars of A. tillyardianum (fig. 3). Only when instar groupings are grossly incorrect are deviations striking enough to be considered indisputably significant.

In the 8 "instars" case, it is difficult to decide if instars 6 and 7 have in fact been lumped. There is an upward deviation for each measurement, but the deviations are not striking. If only one measurement had been graphed, as has been the case when other investigators have used Brooks' rule, and all conclusions had been based on this one measurement, then it is probable that it would be thought that all instars had been successfully separated. Only when all measurements are considered together, and it is seen that all show the same type of deviation at the same point, that suspicions would be aroused that the instar groupings had been incorrectly formed.

In the 7 "instars" case, deviations from a straight line are marked, and it is obvious that the instars have not been correctly separated (fig. 3). However, it is still problematical whether the two "instars" causing the deviations would be correctly regrouped as four instars, particularly if there were no obvious morphological differences between these instars. By using Brooks' rule, though, the researcher would have cause to reconsider his instar groupings, and herein lies the value of

this test.

Therefore, the "growth ratio rule" provides a criterion for defining a significant deviation from a straight line logarithmic progression, and it quantifies the extent of the deviation. The graphical method is less useful because it provides no such criterion, and all decisions are subjective.

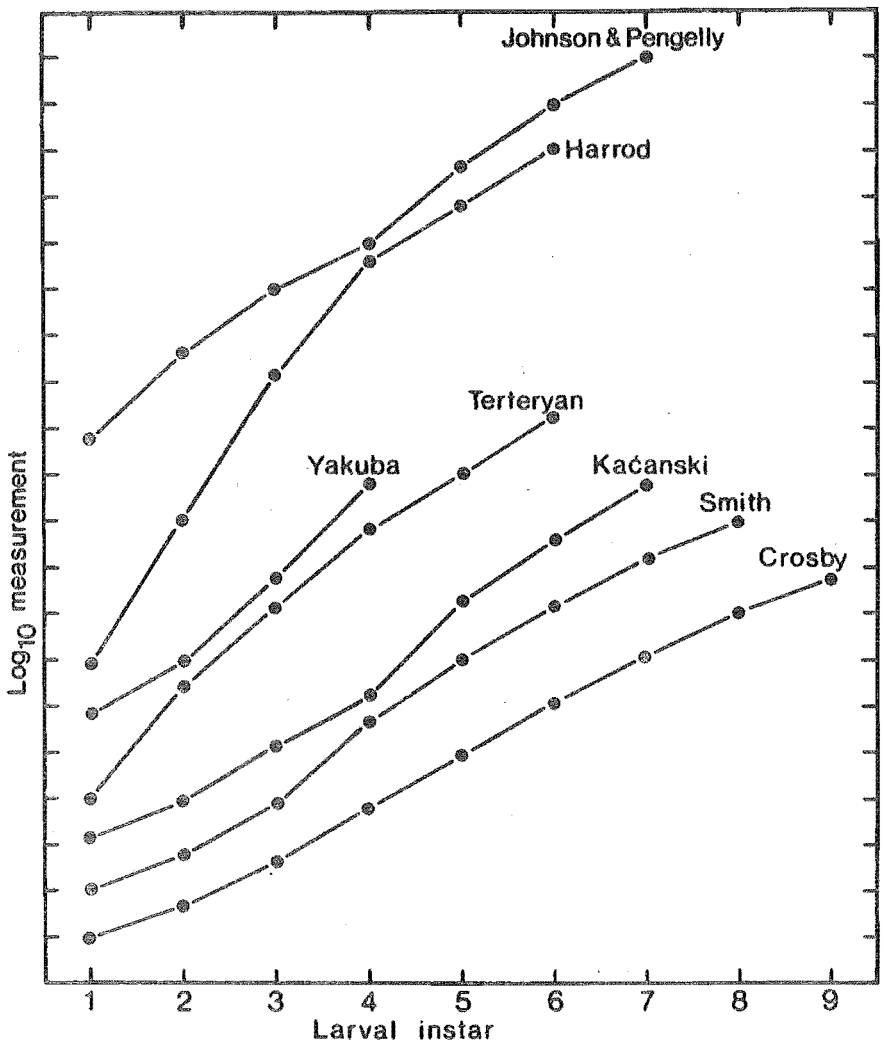



FIG. 4. Plots of logarithmic progressions for head capsule or cephalic apotome widths for simuliid species that have been studied. (Identified according to author).

Application of Brooks' rule to other simuliid studies

Brooks' rule has been used in some other simuliid instar determination studies, but the results of applying the method have been contradictory. In all cases, the rule was applied to only one measurement, either head capsule width or cephalic apotome width (fig. 4 ), and this in itself poses problems of interpretation.

Harrod (1964) considered that the cephalic apotome width of Simulium (Odagmia) nitidifrons did not conform to Brooks' rule, and this is borne out by plotting her results (fig. 4 ) and by applying the growth ratio rule. Although the deviation could suggest that the instars have not been separated correctly, a factor which must influence the result is that growth of the cephalic apotome is not constant from instar to instar; that is, one of the assumptions of Brooks' rule is violated.

In contrast, Johnson & Pengelly (1970) considered that the cephalic apotome width of Simulium (Simulium) rugglesi conformed to Brooks' rule and supported their division into seven instars. A deviation around their instar 5, however, may indicate that the groups are not entirely correct. This supposition is further supported by the results of applying the growth ratio rule which shows that the deviation is significant and there may be another instar.

Smith (unpublished 1969) implied that Brooks' rule can be applied to head capsule width in Prosimulium (Prosimulium) hirtipes, and this certainly appears to be the case. A slight deviation around instar 4 probably indicates little more than a random fluctuation around the logarithmic progression.

Kaćanski (1968), Terteryan (1957) and Yakuba (1960) did not use Brooks' rule in their studies, but their results have been plotted for comparison with the studies where it has been used (fig.4 ). Kaćanski studied Simulium (Odagmia) ornatum, and it would appear as if an instar has been overlooked between her instars 4 and 5, as the deviation is significant according to the growth ratio rule. Terteryan's results do not follow a logarithmic progression at all; either this is indeed the case, possibly indicating seasonal differences, or the instars have been incorrectly grouped. The results of Yakuba follow a logarithmic progression very well, but this is misleading since it is obvious from his paper that the instars have been incorrectly divided. Yakuba's results emphasize the importance of using more than one type of evidence when attempting to determine the number of instars.

To summarize, it is clear that Brooks' rule is of use in checking if an instar series is complete, but it should be used in conjunction with other, preferably morphological, evidence.

## Student's t-test

The purpose of a t-test is to test the location of the means of two samples, and so find if the samples can be considered as coming from the same population or from different populations (Sokal & Rohlf, 1969). It is assumed that the data of both samples are normally distributed.

In this part of the study, the t-test was used as a technique for testing a measurement for statistical differences between two successive instar groups that were morphologically distinct.

Since there was very little overlap of the measurements between instars of A. tillyardianum, it would be expected that there would be significant differences between the mean measurements of the instars. This is so. Nearly all the comparisons gave t-values that were significantly different at the 0.1% level of significance. Only one comparison failed to reveal a significant difference between successive instars, that of antennal segment 4 length between instars 1 and 2; this may be explained by the small size of the structure being measured (8.6 μm), and the small increase between successive instars. Thus, the t-tests confirmed that instar groups morphologically distinct were also biometrically distinct.

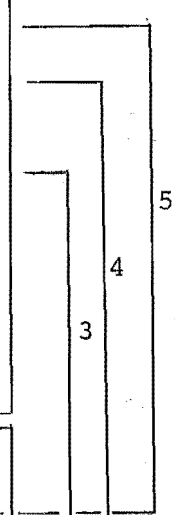
## Sensitivity of the t-test

If instars have been separated on the basis of morphological features, then the t-test is a perfectly valid statistical test to use. On the other hand, if instars have been separated by dividing measurements

TABLE IX

Effect of splitting a measurement into groups without reference to morphological features and then applying t-tests

| "Measurement" | | Group |
|---|---|---|
| Eyepiece graticule units (x100) | µm | |
| 21 | 306.6 | 1 |
| 22 | 321.2 | |
| 23 | 335.8 | |
| 24 | 350.4 | |
| 25 | 365.0 | |
| 26 | 379.6 | |
| 27 | 394.2 | |
| 28 | 408.8 | |
| 29 | 423.4 | |
| 30 | 438.0 | |
| 31 | 452.6 | |
| 32 | 476.2 | |
| 33 | 481.8 | |
| 34 | 496.4 | |
| 35 | 511.0 | |
| 36 | 525.6 | |
| 37 | 540.2 | 2 |
| 38 | 554.8 | |
| 39 | 569.4 | |
| 40 | 584.0 | |




Eyepiece graticule units

| Group | Mean ($\bar{Y}$) |
|---|---|
| 1 | 25.5 |
| 2 | 35.5 |
| 3 | 30.5 |
| 4 | 28.5 |
| 5 | 27.5 |

$N = 10$

$s^2 = 8.25$

$s_{\bar{Y}} = 0.908$

µm units

| Group | Mean ($\bar{Y}$) |
|---|---|
| 1 | 372.3 |
| 2 | 518.3 |
| 3 | 445.3 |
| 4 | 416.1 |
| 5 | 401.5 |

$N = 10$

$s^2 = 1758.57$

$s_{\bar{Y}} = 13.261$

| t-tests between groups | $t$ value (d.f. = 18) | p |
|---|---|---|
| 1 - 2 | 7.785 | < 0.001 *** |
| 1 - 3 | 3.893 | 0.001 - 0.005 ** |
| 1 - 4 | 2.336 | 0.025 - 0.050 * |
| 1 - 5 | 1.557 | 0.1 - 0.2 n.s. |

into segments that are thought to represent instars, then results of the t-tests may be very misleading.

The effect of splitting a measurement into groups without reference to morphological features and applying t-tests to discover if the groups are statistically different is simulated in Table IX . Splitting the continuous measurement scale into non-overlapping groups results in highly significant differences between groups no matter how haphazard the division. It is not until there is almost complete overlap between groups that t-test values suggest that there are no significant differences between the groups (Table IX ).

When a typical bell-shaped curve is split in half, for example, a measurement for one instar, a t-test reveals that there are highly significant differences between the two halves. As in the case above, groups have been truncated to a greater or lesser extent, and, as this violates the assumptions of normally distributed data, the outcome of t-tests would be expected to be affected.

Thus, the t-test can not show if lumping or splitting of instars has occurred; it only shows if the mean values of the instar groups are sufficiently distinct to be statistically different.

TABLE X

Effect of lumping instars on t-test values, standardization set of *A. tillyardianum* data, using instars 4, 5, 6 and 7

| Comparison between instars | t values for variable numbers | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 11 |
| 4 - 5 | 7.5 | 10.7 | 12.8 | 11.4 | 15.7 | 10.8 | 12.0 | 4.6 | 12.8 | 5.2 |
| 5 - 6 | 9.2 | 9.2 | 8.7 | 7.0 | 13.2 | 9.7 | 11.1 | 3.3 | 11.1 | 4.8 |
| 6 - 7 | 10.2 | 12.0 | 9.9 | 9.2 | 11.5 | 10.6 | 7.0 | 5.1 | 9.9 | 4.2 |
| 4 & 5 - 6 & 7 | 17.6 | 17.3 | 17.2 | 15.6 | 19.7 | 18.2 | 17.6 | 9.7 | 18.7 | 11.2 |

Effect of lumping instars on t values

When instar groups of A. tillyardianum are lumped together, and the lumped categories are tested for significant differences between mean values of the measurements, the t values obtained are larger than those obtained when instars are correctly separated (Table X ). Such results show that the t-test is an unsuitable statistical test to use if the t values that are calculated are to be interpreted as indicating whether or not lumping of instar groups has occurred. The t values only reflect the magnitude of the difference between mean measurements of groups, they do not confirm the validity of the groups.

Summarizing, although it is tempting to place undue emphasis upon highly significant statistical differences that may occur between instar groups, or to suggest that highly significant differences between instar groups proves the validity of the instars, only morphological differences between instar groups can be considered to represent "proof" that the instar groups are true. To state that instar groups are statistically different without reference to morphological differences is meaningless, and any interpretations of such results should be regarded with caution.

## Multiple discriminant function analysis

Unlike the univariate Student's t-test in which only one measurement at a time is considered, the multivariate statistical technique of Multiple Discriminant Function analysis (MDFA) considers all measurements simultaneously. The effect of such a technique is that the overall "shape" and dimensions of an instar group is tested for differences with other instar groups, and theoretically this approach should be more efficient than the single measurement approach. As with the t-test, it is assumed that the data are normally distributed, and that the groups have been formed on the basis of predetermined characteristics such as features of morphology. A further restriction is that there can be no missing measurements for any individual.

In MDFA, a set of linear functions is calculated from a standardization set of data on groups to allow the classification of individuals into one of several groups. The functions are calculated so that the within-group variance is minimized and the between-group variance is maximized, allowing the groups to be optimally separated. The classification of an individual is performed by evaluating the calculated functions with the measurements of an individual, and deciding to what group the values of the individual most resemble. Thus, in using linear discriminant functions, original measurements of an individual are recombined to give new values of a purely artificial nature that are only of use in classifying (Bargmann, 1969, 1970). Because of the involved computations, the use of MDFA has become a practical statistical method only recently with the advent of high-speed computers.

There are two main types of discriminant analysis, both of which

were used in this study. The more widely used type is Fisher's linear discriminant function where the data matrices are reduced in dimensionality to one, two or three "significant" linear combinations in which much of the data variation is explained. In contrast, the other type of discriminant function, the quadratic form which is calculated to give a "linear" solution, does not attempt to reduce the dimensionality of the data matrices or to attempt to separate out "significant" linear combinations that explain most of the data variations (Gnadadesikan & Wilk, 1969). In this study, the quadratic form was considered to be the more useful of the two.

The purpose of the analyses was threefold. Firstly, it was to calculate discriminant function equations, and then for each individual of an instar group evaluate the equations and see to what instar the measurements of the individual most resembled. Secondly, it was to use different subsets of measurements to discover the relative effectiveness of different measurements in predicting the instar membership of an individual. Thirdly, it was also hoped that these data explorations would reveal how different individuals were affected by various statistical manipulations of a data set (Tukey, 1969).

The data were not transformed for the discriminant function analyses, although the results of multivariate analysis of variance (MANOVA of Cooley & Lohnes (1971)) between the nine instars using 9 variables suggested that a base 10 logarithmic scale would make the dispersion matrices homogeneous and would enhance the separation of instars. The test on untransformed data for $H_1$, the equality of dispersions, gave an F-value of 1.997 for 360 and 8 186 degrees of freedom, a value indicating that the dispersion matrices were very significantly

different between the 9 instars. When the data were transformed to a $\log_{10}$ scale, the F-value was 0.623, showing that the dispersion matrices were homogeneous. With the untransformed data the test for $H_2$, the overall discrimination between groups, gave an F-value of 22.91 for 72 and 840 degrees of freedom, showing that there was very good separation of the instars. On a $\log_{10}$ scale the F-value was even higher, 58.21, indicating that the separation of the instars had been further enhanced by the new scale.

However, the results of the univariate F-ratios on variances given by the randomization tests suggested that no meaningful interpretations about the homogeneity of variances could be made on the standardization data because of the effects of the measuring scale (p.69). It would seem very likely that analogous results would be obtained for the multivariate counterpart, since the data are the same. Another reason for not transforming the data was because most of the analyses were to calculate discriminant functions that would separate adjacent instars, and adjacent dispersion matrices appeared to be comparable. Also, if the dispersion matrices were heterogeneous, then the variance discrepancies could be utilized by the quadratic discriminant function to give a better separation of the instars (Gilbert, 1969).

Quadratic discriminant functions

Information on this type of discriminant function analysis is scattered. Accounts have been presented by Kendall (1957), Anderson (1958), Dixon (1965) and I.B.M. (1968) on the theory and methods of calculating the functions, whereas Lejebvre & Lennes (1969), Eaton & Lapins (1970) and Knight (in press) have illustrated the biological use of the functions in discriminating between groups. In this study, a modified version of the IBM Application Program MDISC from the System/360 Scientific Subroutine Package was used (I.B.M., 1968).

The quadratic discriminant function equation is of the form

$$z_k = c_{1k} \cdot x_{1k} \pm c_{2k} \cdot x_{2k} \ldots \pm c_{mk} \cdot x_{mk} - c_{0k}$$

where $z_k$ = the discriminant value that is calculated from the $k^{th}$ group discriminant function

$c_{1k}, c_{2k} \ldots c_{mk}$ = the coefficients calculated for the m variables of the $k^{th}$ group

$x_{1k}, x_{2k} \ldots x_{mk}$ = the corresponding m variables of the $k^{th}$ group

$c_{0k}$ = the constant of the $k^{th}$ group

The number of discriminant functions calculated is the same as the number of groups used for the analysis, that is, there is no reduction in dimensionality. Every discriminant function can then be evaluated using the measurements of each individual in turn. For an individual, the group discriminant function which gives the largest discriminant value indicates the group to which an individual most resembles and should be assigned to.

Discriminant runs undertaken

As well as using all 16 variables to distinguish between instars, subsets of these variables were also chosen to test the efficiency of discriminant analysis under different conditions. Choice of these subsets was subjective; some of the variables chosen were those thought to have been accurately measured, and others were those for which the measurements did not appear to be instar characteristic.

In the analyses using all 16 variables, four were dummy variables (numbers 13, 14, 15, and 16) similar to the use of dummy variables in other studies (Dempster, 1969; Eaton & Lapins, 1970). These served to separate the first five instars.

The following runs were undertaken.

A. To discriminate between all 9 instars
   - i) all 16 variables
   - ii) 9 variables, numbers 2, 3, 4, 5, 7, 8, 9, 10, and 11.

B. To discriminate between 2 successive instars
   - iii) selection 1, all 16 variables
     - a) instars 1 & 2, 3 & 4, 5 & 6, 7 & 8.
     - b) instars 2 & 3, 4 & 5, 6 & 7, 8 & 9.
   - iv) selection 2, 9 variables, numbers 2, 3, 4, 5, 7, 8, 9, 10, and 11.
     - a) instars 1 & 2, 3 & 4, 5 & 6, 7 & 8.
     - b) instars 2 & 3, 4 & 5, 6 & 7, 8 & 9.
   - v) selection 3, 3 variables, numbers 4, 5 and 10. These were thought to be the best 3 measurements for separating the instars.
     - a) instars 1 & 2, 3 & 4, 5 & 6, 7 & 8.

b) instars 2 & 3, 4 & 5, 6 & 7, 8 & 9.

vi) selection 4, 2 variables, numbers 5 and 10. These were thought to be the best two measurements for separating the instars.

a) instars 1 & 2, 3 & 4, 5 & 6, 7 & 8.

b) instars 2 & 3, 4 & 5, 6 & 7, 8 & 9.

vii) selection 5, 2 variables, numbers 1 and 2. These were two measurements for which the values overlapped between instars.

a) instars 1 & 2, 3 & 4, 5 & 6, 7 & 8.

b) instars 2 & 3, 4 & 5, 6 & 7, 8 & 9.

viii) selection 6, 2 variables, numbers 4 and 5. These were the two most easily taken measurements.

a) instars 1 & 2, 3 & 4, 5 & 6, 7 & 8.

b) instars 2 & 3, 4 & 5, 6 & 7, 8 & 9.

All the above analyses were carried out with the standardization set of data. The resultant discriminant function equations were evaluated using the measurements of each individual to give discriminant values. Values from each discriminant function equation were analyzed to find the mean discriminant value and variance of the sample. Then the discriminant values of individuals were checked to see if any were outside the 95 or 99% confidence limits of the sample. Such analysis of the data meant that each individual of the standardization set could be tested for membership in the instar group it was assigned to previously by morphological features when using different combinations of measurements which then were the criteria of membership.

Although the discriminant function equations formed from the standardization set of data would be expected to give optimal separation of the instars for these data, it does not follow automatically that these

equations would separate other newly-measured individuals into instar groups with the same precision or certainty; in fact, the equations may have no predictive power for new data (Howells, 1966). Therefore, cross-validation of the standardization equations was carried out by using the two test sets of larvae collected at different times of the year (p.50 ), and seeing if the individuals were correctly assigned to their respective instar groups. This approach is desirable, according to Cooley & Lohnes (1971), since "Prediction on replication samples is protection against capitalization on chance."

Measurements of the test larvae were evaluated in the standardization discriminant function equations and the discriminant values obtained were checked to see if any were outside the 95 or 99% confidence limits of the standardization sample values (Tables XV and XVII ).

As a further check on the sensitivity of MDFA in assigning individuals to groups, the above procedures were repeated using the less variable 3-4-71 test set data as a standardization set, and the standardization set of data as a test set. These results are summarized in Tables XX-XXV.

Results of the discriminant function analyses

Differing numbers of individuals of the standardization set were misassigned to their instar group according to the combination of variables that had been used in the discriminant function equations. These misclassifications are summarized in Table XI , which indicates what individuals were misclassified and to what instar a misclassified individual was assigned to.

TABLE XI

Individuals of the standardization set misclassified by the standardization set of discriminant function equations, and the instar these individuals were misclassified to. An empty space indicates an individual was correctly assigned to instar

| Instar | Individual | Discriminant runs: 16 vars | 9 vars | 1 a | 1 b | 2 a | 2 b | 3 a | 3 b | 4 a | 4 b | 5 a | 5 b | 6 a | 6 b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 6 | | | | | | | 1 | | 1 | | 1 | | 1 | |
| 3 | 15 | | | | 2 | | | | | | 2 | | 2 | | |
| | 16 | | | | | | | | | | | | 2 | | 2 |
| | 17 | | | | | | | | | | | | 2 | | 2 |
| | 18 | 4 | | 4 | | | | | | | | 4 | | | |
| | 19 | | | | | | | | | | | 4 | | | |
| 4 | 9 | | | | | | | | | | | 3 | | | |
| | 10 | 5 | | | 5 | | | | | | | | | | |
| | 11 | 5 | 5 | | 5 | | | | | | | | 5 | | |
| | 13 | | | | | | | | | | | 3 | | | |
| | 14 | | | | | | | | | | | 3 | | | |
| | 16 | | | | | | | | | | | 3 | | | |
| 5 | 1 | 4 | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | | 4 |
| | 3 | 4 | | | 4 | | | | | | 4 | | | | |
| | 7 | | | | 4 | | | | | | | | 4 | | |
| | 10 | | | | | | | | | | | 6 | | | |
| | 13 | 4 | | | 4 | | | | | | | | | | |
| | 16 | | | | | | | | | | | | 4 | | |
| | 19 | 6 | 6 | 6 | | | | | | | | | | | |
| 6 | 3 | | | | | | | | | | | | | | 7 |
| | 4 | 7 | | | 7 | | | | | | | | | | |
| | 7 | | | 5 | | | | | | | | | | | |
| | 10 | | | | | | | | | | | 5 | | | |
| | 16 | | | | | | | | | | | | | 5 | |
| | 17 | | | | | | | | | | | 5 | | 5 | |
| | 18 | | | | | | | | | | | 5 | | | |
| 7 | 1 | | 8 | | | | | | | | | | | 8 | |
| | 2 | | 8 | | | | | | | | | | | 8 | |
| | 7 | | | | | | | | | | | 8 | | | |
| | 9 | | | | | | | | | | | 8 | | | |
| | 12 | 8 | | | | | | | | | | | | | |
| | 13 | 6 | | | 6 | | | | | | | | 6 | | |
| | 17 | | | | | | | | | | | | | | 6 |
| | 18 | 8 | | 8 | | | | | | | | | | | |
| | 20 | | | | | | | | | | | | 6 | | |
| | 21 | | 8 | | | | | 8 | | 8 | | 8 | | 8 | |
| | 22 | | 8 | | | | | | | 8 | | 8 | | 8 | |
| | 23 | 6 | | | 6 | | | | 6 | | 6 | | | | |
| 8 | 1 | | | | | | | | | | | | 9 | | |
| | 4 | | | | | | | | | | | | 9 | | |
| | 9 | | | | | | | | | | | 7 | | | |
| | 15 | | 7 | | | 7 | | | | | | 7 | | | |
| | 18 | | | | | | | | | | | 7 | | | |
| 9 | 9 | | | | 8 | | | | | | | | 8 | | |
| Total misclassified | | 12 | 7 | 4 | 11 | 1 | 1 | 2 | 2 | 3 | 4 | 19 | 12 | 7 | 5 |
| Total correctly classified | | 142 | 147 | 136 | 131 | 139 | 141 | 138 | 140 | 137 | 138 | 121 | 130 | 133 | 137 |

A. Discriminating between 9 instars

When using all 16 variables, 12 out of the 154 individuals were misclassified, a misclassification rate of about 8.0%. The subset of 9 variables resulted in 7 individuals being incorrectly classified, a misclassification rate of only 4.5%. Of these 7 individuals, 4 were those that had been correctly classified using the 16 variables. The improvement in classification by using a subset of variables is surprising. It would appear as if the dummy and morphological variables were of no real use in the analysis as they seemed to nullify rather than improve classification accuracy.

B. Discriminating between 2 instars

Since an individual can be placed in only one of two instars, it would be expected that fewer individuals would be misclassified in these selections: in most instances, this is so.

As in the case of discriminating between 9 instars, the use of 16 variables (selection 1) gave a higher misclassification rate than the subset of 9 variables (selection 2). In fact, in the 16 variable example, more individuals were misclassified with the two instar choice than in the nine instar choice. The opposite was true with the 9 variable example. These results confirm the conclusions above that the dummy and morphological variables were of little use in the discriminant function equations.

The three best variables (selection 3) classified most individuals to their correct instar -- the accuracy of classification

was comparable to using the 9 variable subset. This indicates that some variables in the 9 variable analysis were redundant because of intercorrelations between variables.

In the remaining three selections in which two variables were used, the number of individuals misclassified was related to the subjective assessment of the reliability of the measurements in separating instars. The two measurements thought to separate the instars best (selection 4) separated well, although about twice as many individuals were misclassified as were in selection 3. The two measurements for which the values overlapped between instars (selection 5) resulted in the most individuals being misclassified of all the analyses, but even so the number of individuals successfully classified approached 90%: this analysis demonstrates the power of the discriminant function technique for separating instars. Selection 6, with the two most easily taken measurements (postocciput width and mandible length), proved to be very good for classifying individuals as fewer than 5% were misclassified. Although this is a higher misclassification rate than when using the two best measurements, the easier measuring makes these two measurements the best combination to use.

To summarize, using the discriminant function equations formed from the standardization data, most individuals of the standardization set could be correctly classified to their instar group. Thus, the measurements of an individual quite closely reflect the morphological characters of an individual. It follows then that biometrical differences between instars correspond to morphological differences.

Assignment according to mean discriminant value confidence limits

Before an individual can be placed in a grouping using MDFA, each discriminant function equation has to be evaluated. The equation which gives the highest discriminant value indicates the grouping that the individual most resembles, and to which the individual should be assigned.

However, when there is little overlap between groupings, a single discriminant equation may be sufficient to characterize and place an individual to its grouping. In such a special case, the means of the groups lie on a straight line, and there is an equal probability of an individual belonging to any of the groups. Consequently the discriminant equations calculated for each grouping are proportional to each other, and any one equation may be as powerful as Fisher's linear discriminant function in characterizing the groupings (Kendall, 1957).

The A. tillyardianum instar groups appeared to comply with the conditions of the special case. Therefore, the efficiency of each discriminant equation, as shown by the number of individuals outside the 95 and 99% confidence limits of the discriminant value means of the discriminant equation, was tested in assigning individuals to instar groups in which they had been previously placed on the basis of morphological features (Tables XII and XIII).

Because it is now the discriminant value derived from one equation that is used to assign an individual to an instar rather than the highest discriminant value from a series of equations, it would be expected that this method would be less sensitive in confirming that an individual was a member of its previously assigned instar group. The reason for this is that the range of discriminant values within an instar can be

TABLE XII

Individuals of the standardization set whose discriminant values were outside the confidence limits of the standardization set of discriminant function equations. 95 = 95%, 99 = 99%; underlining indicates a discriminant value outside the confidence limit of the lower tail of a sample distribution

| Instar | Individual | Discriminant runs and equations | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 16 vars | 9 vars | 1 a | | 1 b | | 2 a | | 2 b | | 3 a | | 3 b | | 4 a | | 4 b | | 5 a | | 5 b | | 6 a | | 6 b | |
| | | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 1 | 1 | | 95 | | | | | | | | | | | | | | | | | | | | | | | | |
| | 6 | | | 95 | 95 | | | 95 | 95 | | | | | | | 95 | 95 | | | | | | | | | | |
| | 7 | | | | | | | | | | | | | | | | | | | | | | | 95 | 95 | | |
| 3 | 15 | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 | | | | | | | | |
| | 16 | | | | | | | | | | | | | 95 | 95 | | | | | | | | | | | | |
| | 17 | | | | | | | | | | | | | 95 | 95 | | | | | | | | | | | | |
| | 18 | 95 | | 95 | 95 | 95 | 95 | | | | | | | | | | | | | | | | | | | | |
| 4 | 2 | | | | | | | | | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | | | | 95 | 95 | 95 | 95 |
| | 10 | 95 | | | | 95 | 95 | 95 | | | | | | | | | | | | | | | | | | | |
| | 11 | 95 | 95 | 95 | 99 | 95 | 95 | | | | | | | | | | | | | | | | | | | | |
| 5 | 1 | | | | | | | | | | | | | 95 | 95 | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 |
| | 3 | | | | | | | | | | | | | | | | | 95 | 95 | | | | | | | | |
| | 13 | | 95 | | | | | 95 | 95 | | | | | | | | | | | | | | | | | | |
| | 17 | | | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | |
| 6 | 4 | | | 95 | 95 | | | | | | | | | | | | | | | | | | | | | | |
| 7 | 21 | | | | | | | | | | 95 | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| | 23 | 95 | | 95 | 95 | 95 | 95 | 95 | | 95 | 95 | | | | | | | | | | | | | | | | |
| 8 | 12 | | | | | | | | | | | | | | | | | | | | | | | 95 | 95 | 95 | |
| | 15 | | | | | | | | | | | | | | | | | | | | | 95 | 95 | | | | |

TABLE XIII

Number of individuals of standardization set in the standardization set of discriminant function equations for which their discriminant values were outside the confidence limits of the instar they had been assigned to. The diagonal represents the characteristic equation for each instar, and corresponds to the equations used in Table XII for the 16 and 9 variable cases. Columns are equations, showing how many individuals were misassigned in each instar. Rows are instars, showing how many individuals were misassigned in each equation (usually the same individuals were misassigned by each equation)

a) discriminant equations separating 9 instars using 16 variables

| Instar | Instar equation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | |
| 4 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Total | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 5 | 6 | 4 |

b) discriminant equations separating 9 instars using 9 variables

| Instar | Instar equation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 3 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | |
| 4 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | |
| 5 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | |
| 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 7 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| 9 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | |
| Total | 4 | 5 | 5 | 5 | 5 | 6 | 4 | 4 | 4 | 3 |

wide, particularly when the measurements used for the equations overlap between instars. As a result, confidence intervals around the mean of an instar may be sufficiently wide to include adjacent instars. Therefore, measurements of an individual may have to be well outside the dimensions of its instar group before it would be detected as possibly not being a member of its assigned instar group.

Comparison of Tables XI and XII confirms this supposition. More individuals are considered to be incorrectly placed to their instar group when the largest discriminant value from a series of equations is used as the criterion of membership than when the discriminant value from one equation is used. Generally, the less overlap there is of measurements between instars, the greater the correspondence between the number of individuals misassigned. Thus, the main difference between Tables XI and XII is selection 5, in which the two measurements overlap between instars. For example, in selection 5a (Table XI ) 19 individuals were misassigned when the larger of the two discriminant values was used as criterion of membership, but only 2 individuals were considered to be misassigned when using the discriminant value of a single equation (Table XII).

In Table XII, results of the 16 and 9 variable cases to separate the 9 instars are those obtained by using the characteristic equation for each instar only, and not by using one of the nine possible equations. When a single equation is used, the accuracy of placing an individual to an instar is nearly equivalent to using the characteristic equations (Table XIII), although some different individuals are misassigned. It does not appear to matter what equation is chosen since they all yield similar results.

TABLE XIV

Individuals of the 3-4-71 test set misclassified by the standardization set of discriminant function equations, and the instar these individuals were misclassified to. An empty space indicates an individual was correctly assigned to instar

| | | Discriminant runs | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Instar | Individual | 16 vars | 9 vars | 1 a | 1 b | 2 a | 2 b | 3 a | 3 b | 4 a | 4 b | 5 a | 5 b | 6 a | 6 b |
| 2 | 1 | 1 | | | | 1 | | | | | | | | | 3 |
| | 2 | 1 | | | | 1 | | | | 1 | | | | | |
| | 3 | 1 | | | | | | | | | | | | | |
| | 4 | 1 | | | | | | | | | | | | | |
| | 5 | 1 | | | | | | | | | | | | | |
| | 6 | 1 | | | | 1 | | | | | | | | | |
| | 7 | 1 | | | | | 3 | | | | | | | | |
| | 8 | 1 | | | | | | | | | | | | | |
| | 9 | 1 | | | | | | | | | | | | | |
| | 10 | 1 | | | | | | | | | | | | | |
| 3 | 1 | 2 | | | | | | | | | | | | | |
| | 2 | | | | | | | | | | 2 | | | | |
| | 3 | | | | | | | | | | | 4 | | | |
| | 8 | | | | | | | | | | | 4 | | | |
| | 9 | | | | | | | | | | | 4 | | | |
| | 10 | | | | | | | | | | | 4 | | | |
| 4 | 2 | 3 | | | | | | | | | | | | | |
| | 3 | 3 | | | | | | | | | | | | | |
| | 4 | 3 | | | | | | | | | | | | | |
| | 5 | 3 | | | | | | | | | | | | | |
| | 6 | 3 | | | | | | | | | | | 5 | | |
| | 7 | 3 | | | | | | | | | | | 5 | | |
| | 8 | | | | | | 5 | | 5 | | | | 5 | | |
| | 9 | | | | | | | | | | | | 5 | | |
| 6 | 1 | 5 | | 5 | | | | | | | | | | | |
| | 4 | 5 | | | | 5 | | | | | | 5 | | | |
| | 6 | 5 | 5 | 5 | | 5 | | 5 | | 5 | | 5 | | 5 | |
| | 7 | 5 | 5 | 5 | | 5 | | 5 | | 5 | | 5 | | 5 | |
| 7 | 1 | 6 | | | 6 | | | | 6 | | 6 | | | | 6 |
| | 2 | 6 | 6 | | 6 | | | | 6 | | 6 | | | | 6 |
| | 4 | 6 | | | 6 | | | | 6 | | 6 | | | | 6 |
| | 6 | 6 | 6 | | 6 | | 6 | | 6 | | 6 | | | | 6 |
| | 7 | 6 | | | 6 | | | | | | | | | | |
| | 8 | 6 | 6 | | 6 | | 6 | | 6 | | 6 | | | | 6 |
| | 9 | 6 | | | 6 | | | | | | | | | | |
| | 10 | 6 | | | 6 | | | | 6 | | 6 | | | | 6 |
| 8 | 1 | 7 | 7 | | | | | 7 | | 7 | | | | 7 | |
| | 2 | 7 | 7 | 7 | | 7 | | 7 | | 7 | | | | 7 | |
| | 3 | | 7 | | | | | | | | | | | 7 | |
| | 4 | 7 | | | | | | | | | | | | | |
| | 5 | 7 | 7 | 7 | | 7 | | 7 | | 7 | | | | 7 | |
| | 6 | 7 | | 7 | | | | | | | | | | | |
| 9 | 1 | | | | | | | | | | | | 8 | | 8 |
| | 2 | 8 | 8 | | 8 | | 8 | | 8 | | 8 | | | | 8 |
| | 3 | 8 | 8 | | 8 | | | | 8 | | 8 | | 8 | | 8 |
| | 5 | | 8 | | | | 8 | | | | | | | | |
| | 7 | | 8 | | | | | | | | | | | | 8 |
| | 8 | | 8 | | | | | | | | | | | | |
| | 9 | | 8 | | | | 8 | | | | | | 8 | | 8 |
| | 10 | 8 | 8 | | 8 | | | | | | | | 8 | | |

TABLE XV

Individuals of the 3-4-71 test set whose discriminant values were outside the confidence limits of the standardization set of discriminant function equations. 95 = 95%, 99 = 99%; underlining indicates a discriminant value outside the confidence limit of the lower tail of a sample distribution

| Instar | Individual | 16 vars | 9 vars | Discriminant runs and equations | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | | | | 2 | | | | 3 | | | | 4 | | | | 5 | | | | 6 | | | |
| | | | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | |
| | | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 1 | 2 | | | 95 | 95 | | | 95 | 95 | | | | | | | 95 | 95 | | | | | | | | | | |
| | 4 | | | 95 | 95 | | | 95 | 95 | | | | | | | | | | | | | | | | | | |
| | 5 | 95 | | | | | | | | | | 95 | 95 | | | 95 | 95 | | | | | | | | | | |
| | 6 | | | | | | | | | | | 95 | 95 | | | 95 | 95 | | | | | | | | | | |
| | 7 | | | | | | | | | | | 95 | 95 | | | 95 | 95 | | | | | | | 95 | 95 | | |
| | 9 | | 95 | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | 1 | 99 | | | | 95 | 95 | 95 | 95 | 99 | 95 | | | 95 | 95 | | | | | | | | | 95 | 95 | 95 | 95 |
| | 2 | 99 | | 99 | 99 | | | 99 | 99 | | | | | | | | | | | | | | | | | | |
| | 3 | 95 | 95 | | | 99 | 95 | | | 95 | | | | | | | | | | | | | | | | | |
| | 4 | 99 | | | | | | 95 | | | | | | | | | | | | | | | | | | | |
| | 5 | 99 | | | | | | 95 | 95 | | | | | | | | | | | | | | | | | | |
| | 6 | 99 | | | | | | 95 | 95 | | | | | | | | | | | | | | | | | | |
| | 7 | 95 | | | | 99 | 99 | | | | | | | | | | | | | | | | | | | | |
| | 8 | 95 | | | | 95 | 95 | | | | | | | | | | | | | | | | | | | | |
| | 9 | 95 | 95 | | | 95 | 95 | | | 95 | | | | | | | | | | | | | | | | | |
| | 10 | 99 | | | | 95 | 95 | | | | | | | | | | | | | | | | | | | | |
| 3 | 1 | 95 | | | | | | | | 95 | | | | | | | | | | | | | | | | | |
| | 2 | | | | | | 95 | | | | | | | | | | | | | | | | | | | | |
| | 3 | | | | | | | | | 95 | | | | | | | | | | | | | | | | | |
| | 5 | | | | | | | | | 95 | | | | | | | | | | | | | | | | | |
| | 6 | | | | | | | | | 95 | | | | | | | | | | | | | | | | | |
| | 8 | | | | | | | 95 | | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 |
| | 10 | | | 95 | 95 | 95 | 95 | | | | | | | | | | | | | | | | | | | | |
| 4 | 1 | | | | | | | 99 | 99 | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 |
| | 2 | | | | | | | 99 | 99 | 99 | 95 | | | 95 | 95 | | | | | | | | | | | | |
| | 3 | | | | | | | | 95 | | | | | | | | | | | | | | | | | | |
| | 4 | | | | | | | 95 | 95 | | | | | | | 95 | 95 | 95 | 95 | | | | | | | | |
| | 6 | | | | | | | | | | 95 | | | | | | | | | 99 | 99 | 95 | 95 | | | | |
| | 7 | | | | | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| | 8 | | | 95 | | | | 99 | 99 | | | 99 | 99 | 99 | 99 | 95 | 95 | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 9 | | | | | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| | 10 | | | | | | | 95 | | | | | | | | | | | | | | | | | | | |
| 5 | 2 | | | | | | | | | 95 | | | | | | | | | | | | | | | | | |
| 6 | 1 | 95 | | 95 | 95 | 95 | 95 | | | | | | | | | | | | | | | | | | | | |
| | 6 | 99 | | 99 | 99 | 99 | 99 | | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | | | | |
| | 7 | 95 | | 95 | 95 | 95 | 95 | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 |
| 7 | 1 | 95 | | | | | | | | | | | | | | | | | | | | | | | | 95 | 95 |
| | 2 | | 95 | 95 | 95 | 95 | 95 | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | | | |
| | 4 | | | | | | | | | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | | 95 | 95 |
| | 6 | 95 | | 95 | 99 | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | 99 | 99 | 95 | 95 |
| | 8 | 95 | | 95 | 95 | 95 | 95 | 95 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | | | |
| | 9 | 95 | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 10 | 95 | | 95 | 95 | 95 | 95 | 95 | 95 | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | | | |
| 8 | 1 | | 99 | | | | | 95 | 95 | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 99 | 99 | 99 | 99 |
| | 2 | 95 | 99 | | | | | 95 | 95 | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 |
| | 3 | | | | | | | | | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 |
| | 4 | | 95 | | | | | 95 | | 99 | 99 | | | | | | | | | | | | | | | | |
| | 5 | 99 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | 95 | 95 | 95 | 95 |
| | 6 | 95 | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 7 | | | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | |
| 9 | 1 | | | | | | | | | 99 | 99 | | | | | | | | | | | | | | | 95 | 95 |
| | 2 | 95 | 99 | | | | | | | 99 | 99 | | | 95 | 95 | | | 95 | 95 | | | | | | | 95 | 95 |
| | 3 | 95 | 99 | | | | | | | 99 | 99 | | | 95 | 95 | | | 95 | 95 | | | | | | | | |
| | 5 | | | | | | | | | 99 | 99 | | | | | | | | | | 95 | | | | | | |
| | 6 | | | | | | | | | | | | | | | | | | | | 95 | 95 | | | | | |
| | 7 | | | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | |
| | 9 | | 95 | | | | | | | 99 | 99 | | | | | | | | | | | | | | | | |

Cross-validation of standardization discriminant equations

There are two main methods of cross-validating discriminant equations; firstly, by dividing a standardization set of data into two, forming discriminant equations and values from one half of these data and then seeing if the calculated discriminant values of the other half of the data are similar (Eaton & Lapins, 1970), and secondly, by using independent test sets of data. In this study, the theoretically sounder method of using independent test sets of data was employed.

When individuals of the test sets were evaluated with the standardization discriminant equations, the discriminant values obtained indicated that the equations did have some predictive power. Most individuals were assigned to the instar groups that they had been placed in on the basis of their morphological features. Tables XIV and XVI, XV and XVIIindicate those individuals of the test sets that were misassigned by the equations. Conclusions about the relative efficiency of using either the largest discriminant value from a full set of equations or of using only a single equation were similar to those derived from consideration of the standardization set of data (p.107 ).

Although about 60% of the individuals were misassigned by one or more equations, as opposed to about 30% of the standardization individuals in the same equations (Table XVIII), this misassignment rate does not invalidate the usefulness of the equations. When the number of equations indicating misassignment of individuals is expressed as a percentage of the total number of equations evaluated, a more realistic classificatory efficiency is obtained (Table XIX ). About 95% of the cases were correctly assigned when the largest discriminant

TABLE XVI

Individuals of the 26-9-71 test set misclassified by the standardization set of discriminant function equations, and the instar these individuals were misclassified to. An empty space indicates an individual was correctly assigned to instar

| Instar | Individual | Discriminant runs: 16 vars | 9 vars | 1 a | 1 b | 2 a | 2 b | 3 a | 3 b | 4 a | 4 b | 5 a | 5 b | 6 a | 6 b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 11 | 1 | | | | | 3 | | | | | | | | |
| | 12 | 1 | | | | | | | | | | | | | |
| | 13 | 1 | | | | | 3 | | | | | | | | |
| | 14 | 1 | | | | | | | | | | | | | |
| | 15 | 1 | | | | | | | | | | | 3 | | |
| 3 | 11 | | | | | | | | | | | 4 | | | |
| | 12 | | | | | | | | | | | 4 | | | |
| | 13 | | | | | | | | | | | 4 | | | |
| | 15 | | | | | 4 | | 4 | | | | | | 4 | |
| 4 | 11 | 3 | | | | | | | | | | | | | |
| | 12 | | | | | | 5 | | | | | | 5 | | |
| | 13 | | | | | | | | | | | | 5 | | |
| | 15 | 3 | | | | | | | | | | | | | |
| 6 | 12 | 5 | | | | | | | | | | | | | |
| | 14 | 5 | | 5 | | | | | | | | | | | |
| 7 | 11 | 6 | | | 6 | | 6 | | 6 | | | | | | |
| | 12 | 6 | | | 6 | | | | 6 | | 6 | | | | |
| | 13 | 6 | 6 | | 6 | | 6 | | 6 | | 6 | | | | 6 |
| | 14 | 6 | | | 6 | | | | | | | | | | |
| | 15 | 6 | | | 6 | | | | | | | 8 | | | |
| 8 | 12 | | | | | | | | | | | | 9 | | |
| | 13 | 7 | | 7 | | 7 | | | | 7 | | | | | |
| | 14 | 7 | | | | | | | | | | | | | |
| | 15 | | 7 | | | 7 | | 7 | | 7 | | | | 7 | |
| 9 | 15 | | 8 | | | | | | 8 | | | | | | 8 |

TABLE XVII

**Individuals of the 26-9-71 test set whose discriminant values were outside the confidence limits of the standardization set of discriminant function equations. 95 = 95%, 99 = 99%; underlining indicates a discriminant value outside the confidence limit of the lower tail of a sample distribution**

| Instar | Individual | 16 vars | 9 vars | Discriminant runs and equations | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | | | | 2 | | | | 3 | | | | 4 | | | | 5 | | | | 6 | | | |
| | | | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | |
| | | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 1 | 11 | | | | | | | | | | | 95 | 95 | | | 95 | 95 | | | | | | | | | | |
| 2 | 11 | 99 | | | | 95 | 99 | | | | | | | | | | | | | | | | | | | | |
| | 12 | 99 | | | | 95 | 95 | | | 99 | 95 | | | 95 | 95 | | | | | | | | | 95 | 95 | 95 | 95 |
| | 13 | 99 | | | | 95 | 95 | | | | | | | | | | | | | | | | | | | | |
| | 14 | 95 | | | | 99 | 95 | | | | | | | | | | | | | | | | | | | | |
| | 15 | 95 | | | | 99 | 99 | | | | | | | | | | | | | | | | | | | | |
| 3 | 11 | | | | | | | 99 | 99 | 99 | 99 | 95 | 95 | 99 | 99 | | | | | | | | | 95 | 95 | 95 | 95 |
| | 12 | | | | | | | | | 95 | | | | | | | | | | | | | | | | | |
| | 15 | | | | | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | 95 | | | | | | 99 | 99 | 99 | 99 |
| 4 | 12 | | | | | | | 99 | 99 | | | 99 | 99 | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 99 | 99 | 99 | 99 |
| | 13 | | | | | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| | 14 | | 95 | | | | | 99 | 99 | | | 99 | 95 | 95 | 95 | | | | | | | | | 99 | 95 | 95 | 95 |
| | 15 | | | | | | | 95 | | | | | | | | | | | | | | | | | | | |
| 5 | 12 | | | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | |
| 6 | 14 | | 95 | 95 | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | 11 | 95 | | | 95 | | | | 95 | | 95 | | | | | | | | | | | | | | | | |
| | 12 | 95 | 95 | 95 | 95 | 95 | 95 | | | | 95 | | | | | | | | | | | | | | | | |
| | 13 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | | 95 | 95 | 95 |
| 8 | 12 | | | | | | | | | 99 | 99 | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| | 13 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | | | |
| | 15 | 95 | | | | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 99 | 99 | 99 | 99 | | | | | 99 | 99 | 99 | 99 |
| 9 | 11 | | | | | | | | | | | | | | | | | | | | | 99 | 99 | | | | |
| | 12 | | | | | | | | | | | | | | | | | | | | | 95 | 95 | | | | |
| | 13 | | | | | | | | | | | | | | | | | | | | | 99 | 99 | | | | |
| | 14 | | | | | | | | | 99 | 99 | | | | | | | | | | | 95 | 95 | | | | |
| | 15 | 95 | | | | | | | | | 95 | | | | | | | | | | | | | | | | |

TABLE XVIII

Percentage of individuals of the three data sets misclassified by one or more of the discriminant function equations

| | Standardization set | | Test set 3-4-71 as standardization set | |
|---|---|---|---|---|
| | Misassigned | Outside confidence limits of discriminant values | Misassigned | Outside confidence limits of discriminant values |
| Standardization set | 29 | 12 | 55 | 82 |
| Test set 3-4-71 | 56 | 63 | 7 | 5 |
| Test set 26-9-71 | 56 | 58 | 13 | 69 |

TABLE XIX

Number of individuals misclassified as a percentage of the total number of classifications evaluated by the discriminant function equations

| | Standardization set | | Test set 3-4-71 as standardization set | |
|---|---|---|---|---|
| | Misassigned | Outside confidence limits of discriminant values | Misassigned | Outside confidence limits of discriminant values |
| Standardization set | 2.1 | 2.2 | 5.8 | 35.0 |
| Test set 3-4-71 | 5.7 | 18.1 | 0.5 | 0.7 |
| Test set 26-9-71 | 4.3 | 16.3 | 0.9 | 16.5 |

value from the appropriate discriminant equations was used as the criterion of membership, whereas about 83% of the discriminant values were inside the confidence limits calculated from the standardization discriminant values. It would appear then that the predictive power of the standardization discriminant equations is similar to that found for discriminant equations in some previous studies (Giles & Elliot, 1962; Howells, 1966; Wilson et al., 1967; Booth & Freedman, 1970; Rightmire, 1970), but better than the predictive power of other studies (Chisci & Martin, 1966; Defries-Gussenhoven, 1966; Merrett, 1966; Wu, 1966; Kendell & Gourlay, 1970).

As well, seasonal differences may account for some of the misassignments, since the discriminant values of many individuals of the larger instars of the 3-4-71 test set were lower than expected, showing that larvae of the 3-4-71 test set were smaller than larvae of the standardization set. These seasonal differences are discussed later (p.133 ).

Test set 3-4-71 data as standardization set

All the foregoing calculations were repeated using the less variable data of the 3-4-71 test set (set 2) as a standardization set, and the standardization set of data (set 1) as a test set. Results of this series of calculations are presented in Tables XX to XXV .

Fewer set 2 individuals were misclassified when using discriminant function equations formed from the set 2 data than when using equations of the set 1 data. This is not surprising since the discriminant function equations were those calculated to give optimal separation of these individuals into instars. On the other hand, more individuals of the set 1 data were misassigned than when using their characteristic equations, the misassignment rate being about equivalent to that of the set 2 data in the set 1 equations. Fewer individuals of the 26-9-71 test set were misassigned in the set 2 equations than in the set 1 equations; it would appear as if the set 2 equations have better predictive power for these individuals than the set 1 equations (Tables XVI & XXIV).

However, on the basis of the percentage number of individuals outside the confidence limits of the set 2 discriminant values, more individuals of the 26-9-71 test set were misassigned by the set 2 equations than the set 1 (Tables XVII & XXV ). This apparent contradiction is a result of the smaller range of values of discriminant values for set 2 data than set 1. Consequently, set 2 variances were smaller and hence confidence intervals of sample values were narrower. An individual of the 26-9-71 test set, although correctly assigned to its instar group on the basis of evaluating discriminant equations,

TABLE XX

Individuals of the 3-4-71 test set misclassifed by the 3-4-71 test set of discriminant function equations, and the instar these individuals were misclassified to. An empty space indicates an individual was correctly assigned to instar

| Instar | Individual | Discriminant runs: 16 vars | 9 vars | 1 a | 1 b | 2 a | 2 b | 3 a | 3 b | 4 a | 4 b | 5 a | 5 b | 6 a | 6 b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 2 | | | | | | | | | | | | | 3 | |
| | 8 | | | | | | | | | | | | 5 | | |
| 6 | 5 | 7 | | | 7 | | | | | | 7 | | | | |
| | 6 | 5 | | 5 | | | | 5 | | 5 | | | | | |
| | 7 | | | | | | | | | 5 | | | | 5 | |
| 7 | 6 | | | | | | | | 6 | | | | | | 6 |

TABLE XXI

Individuals of the 3-4-71 test set whose discriminant values were outside the confidence limits of the 3-4-71 test set of discriminant function equations. 95 = 95%, 99 = 99%; underlining indicates a discriminant value outside the confidence limits of the lower tail of a sample distribution

| Instar | Individual | Discriminant runs and equations | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 16 vars | 9 vars | 1 | | | | 2 | | | | 3 | | | | 4 | | | | 5 | | | | 6 | | | |
| | | | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | |
| | | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 5 | 1 | 95 | | 95 | 95 | 95 | 95 | | | | | | | | | | | | | | | | | | | | |
| | 8 | | | | | | | | | | | 95 | 95 | | | | | | | | | | | 95 | 95 | 95 | 95 |
| 6 | 5 | | | | | | 95 | | | | | | | | | | | | | | | | | | | | |
| | 6 | | | | | 95 | 99 | | | | | | | | | | | | | | | | | | | | |

TABLE XXII

Individuals of the standardization set misclassified by the 3-4-71 test set of discriminant function equations, and the instar these individuals were misclassified to. An empty space indicates an individual was correctly assigned to instar

| Instar | Individual | Discriminant runs: 16 vars | 9 vars | 1 a | 1 b | 2 a | 2 b | 3 a | 3 b | 4 a | 4 b | 5 a | 5 b | 6 a | 6 b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 4 | | | | | | | | | | | 1 | | | |
| | 6 | | | | | | | 1 | | 1 | | | | 1 | |
| | 7 | | | | | | | | | | | 1 | | | |
| 3 | 1 | | | | | | | | | | | | 2 | | |
| | 6 | | | | | | | | | | | | 2 | | |
| | 7 | | | | | | | | | | | | 2 | | |
| | 10 | | | | | | | | | | | | 2 | | 2 |
| | 15 | 2 | | | | | 2 | | 2 | | 2 | | 2 | | 2 |
| | 16 | | | | | | | | 2 | | 2 | | 2 | | 2 |
| | 17 | | | | | | | | 2 | | | | 2 | | 2 |
| | 18 | 4 | 4 | | | | | | | | | | | | |
| 4 | 2 | | | | | | | | | | | 3 | | | |
| | 8 | | | | | | | | | | | 3 | | | |
| | 10 | 5 | | | 5 | | 5 | | 5 | | 5 | | | | |
| | 11 | 5 | 5 | | 5 | | 5 | | 5 | | 5 | | | | |
| | 12 | | | | | | | | | | | 3 | | | |
| | 13 | | | | | | | | | | | 3 | | | |
| | 14 | | | | | | | | | | | 3 | | | |
| | 15 | | | | | | | | | | | 3 | | | |
| | 16 | | | | | | | | | | | 3 | | | |
| | 18 | | | | | | | | | | | 3 | | | |
| 5 | 1 | 4 | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | | 4 |
| | 3 | | | | | | | | 4 | | 4 | | 4 | | 4 |
| | 5 | | | | | | | | | | | | 4 | | |
| | 6 | | | | | | | | | | | | 4 | | |
| | 7 | | | | | | | | | | | | 4 | | |
| | 8 | | 6 | | | 6 | | | | | | | | | |
| | 10 | | 6 | | | | | 6 | | 6 | | 6 | | 6 | |
| | 11 | | | 6 | | | | | | | | | | | |
| | 12 | 6 | | 6 | | 6 | | 6 | | 6 | | | | | |
| | 13 | 4 | 4 | | | | 4 | | 4 | | 4 | | | | |
| | 15 | | | 6 | | | | | | | | | | | |
| | 16 | | | 6 | | | | | | | | | 4 | | |
| | 17 | 6 | | 6 | | 6 | | 6 | | 6 | | | | 6 | |
| | 19 | 6 | 6 | 6 | | | | 6 | | 6 | | | | | |
| | 23 | 6 | | 6 | | 6 | | | | | | | | | |

TABLE XXII- continued

| | | 16 vars | 9 vars | 1 a | 1 b | 2 a | 2 b | 3 a | 3 b | 4 a | 4 b | 5 a | 5 b | 6 a | 6 b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 1 | 7 | 7 | | 7 | | 7 | | 7 | | 7 | | | | 7 |
| | 2 | 7 | | | 7 | | 7 | | 7 | | 7 | | | | 7 |
| | 3 | | | | | | | | | | 7 | | | | 7 |
| | 4 | 7 | | | 7 | | | | 7 | | 7 | | | | |
| | 5 | | | | | | 7 | | 7 | | 7 | | | | 7 |
| | 6 | 7 | | | 7 | | | | 7 | | 7 | | | | 7 |
| | 7 | | | | | | 7 | | | | | | | | 7 |
| | 8 | | | | 7 | | | | | | | | | | |
| | 9 | 7 | | | 7 | | | | 7 | | 7 | | | | |
| | 10 | | 5 | | | | | | | | | 5 | | | |
| | 12 | | | | 7 | | | | | | | | | | |
| | 14 | 7 | 7 | | 7 | | | | 7 | | 7 | | | | 7 |
| | 17 | | 5 | | 7 | | | | | | | 5 | | | |
| 7 | 1 | 8 | 8 | 8 | | 8 | | 8 | | 8 | | | | 8 | |
| | 2 | 8 | 8 | 8 | | 8 | | 8 | | 8 | | | | 8 | |
| | 3 | 8 | 8 | 8 | | | | 8 | | 8 | | 8 | 6 | 8 | |
| | 4 | 8 | | 8 | | | | 8 | | 8 | | | | 8 | |
| | 5 | 8 | 8 | 8 | | | | 8 | | 8 | | | | 8 | |
| | 6 | | | | | | | 8 | | 8 | | | | 8 | |
| | 7 | 8 | 8 | 8 | | | | 8 | | 8 | | 8 | | | |
| | 8 | 8 | 8 | 8 | | | | 8 | | 8 | | | | 8 | |
| | 9 | 8 | 8 | 8 | | 8 | | 8 | | 8 | | 8 | | | |
| | 10 | 8 | | 8 | | | | 8 | | 8 | | | | | |
| | 11 | 8 | 8 | 8 | | | | 8 | | 8 | | | | 8 | |
| | 12 | 8 | | 8 | | | | 8 | | 8 | | | | | |
| | 13 | | | | | | | | | | | | 6 | | |
| | 14 | | | | | | | | | | | | 6 | | |
| | 15 | 8 | | 8 | | | | | | | | | | | |
| | 18 | 8 | | 8 | | | | | | 8 | | | | | |
| | 19 | 8 | | 8 | | | | | | | | | | | |
| | 21 | 8 | 8 | 8 | | 8 | | 8 | | 8 | | 8 | | 8 | |
| | 22 | 8 | 8 | 8 | | 8 | | 8 | | 8 | | 8 | | 8 | |
| | 23 | | | | 6 | | | | | | | | | | |
| 8 | 2 | 9 | | | | | | | | | | | | | |
| | 3 | | 9 | | | | | | 9 | | 9 | | 9 | | 9 |
| | 4 | | | | | | | | 9 | | 9 | | 9 | | 9 |
| | 12 | | 9 | | | | 9 | | 9 | | 9 | | | | 9 |
| | 15 | | | | | 7 | | | | | | 7 | | | |
| | 18 | | 9 | | | 7 | | | | | | 7 | | | |
| 9 | 1 | | | | | | | | | | | | 8 | | |
| | 4 | | | | | | | | | | | | 8 | | |
| | 6 | | | | | | | | | | | | 8 | | |
| | 7 | | | | | | | | | | | | 8 | | |
| | 9 | | | | | | | | | | | | 8 | | |
| | 10 | | | | | | | | | | | | 8 | | |
| | 11 | | | | | | | | | | | | 8 | | |
| | 12 | | | | | | | | | | | | 8 | | |
| | 13 | | | | | | | | | | | | 8 | | |
| | 14 | | | | | | | | | | | | 8 | | |

TABLE XXIII

Individuals of the standardization set whose discriminant values were outside the confidence limits of the 3-4-71 test set of discriminant function equations. 95 = 95%, 99 = 99%; underlining indicates a discriminant value outside the confidence limit of the lower tail of a sample distribution

| Instar | Individual | 16 vars | 9 vars | Discriminant runs and equations | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | | | | 2 | | | | 3 | | | | 4 | | | | 5 | | | | 6 | | | |
| | | | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | |
| | | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 1 | 1 | 99 | | 95 | 95 | | | 99 | 99 | | | | | | | | | | | | | | | | | | |
| | 2 | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | | | |
| | 5 | 95 | | | | | | 95 | 95 | | | | | | | | | | | | | | | | | | |
| | 6 | 99 | | | | | | 99 | 99 | | | 99 | 95 | | | 99 | 95 | | | 95 | 95 | | | | | | |
| | 9 | | | | | | | | | | | | | | | | | | | 95 | 95 | | | | | | |
| | 11 | | | | | | | 95 | 95 | | | | | | | | | | | 99 | 99 | | | | | | |
| | 12 | 95 | | | | | | 99 | 99 | | | | | | | | | | | | | | | | | | |
| 2 | 2 | | | | | | | | | | | 95 | 95 | | | 95 | 95 | 95 | 95 | | | | | 95 | | | |
| | 3 | | | | | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| | 4 | | 95 | 95 | 95 | | | | | 95 | | | | | | | | | | 99 | 99 | 95 | 95 | | | | |
| | 6 | | | | | | | | | | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | 99 | 99 | 99 | 99 |
| | 7 | | 99 | | | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| 3 | 1 | | | | | | | | | 95 | 99 | | | | | | | | | | | 95 | 95 | | | | |
| | 3 | | | | | | | | | 95 | | | | | | | | | | | | | | | | | |
| | 5 | | | | | | | | | 95 | | | 95 | | | | | | | | | | | | | 95 | 95 |
| | 6 | | | | | | | | | 95 | 95 | | | | | | | | | | | 95 | 95 | | | | |
| | 7 | | 95 | | | | | | | 99 | 99 | | 95 | 95 | | | | | | 95 | | 95 | 95 | 95 | 95 | 95 | 95 |
| | 8 | | 99 | 95 | 95 | 95 | 95 | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 9 | | 95 | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 10 | | 99 | | | | | 95 | | 99 | 99 | | 99 | 99 | | | 99 | 99 | | | | 95 | 95 | 95 | 99 | 99 | 99 |
| | 11 | | 95 | | | | | | | 99 | 99 | | | | | | | | | | | | | | | | |
| | 12 | | 99 | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 15 | | 99 | | | | | | | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 16 | | 99 | | | | | 95 | 95 | 99 | 99 | 95 | 95 | 99 | 99 | | | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 17 | | 99 | | | | | 95 | 95 | 99 | 99 | 95 | 95 | 99 | 99 | | | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 18 | 95 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | 95 | 95 | | | | | | | | | | |
| | 19 | | | | | | | 99 | 95 | 99 | 99 | | | 95 | 95 | | | 95 | 95 | | | | | | | 95 | 95 |
| 4 | 8 | 95 | | 95 | 95 | 95 | 95 | | | | | | | | | | | | | | | | | | | | |
| | 9 | | | | | | | | | | | | | | | | | | | 99 | 99 | 99 | 99 | | | | |
| | 10 | 99 | | 99 | 99 | 99 | 99 | | 95 | 99 | 99 | | | 99 | 99 | 95 | 95 | 99 | 99 | | | | | | | | |
| | 11 | 99 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | | | | |
| | 12 | | | | | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| | 13 | | | | | | | | | | | 95 | | | | | | | | 99 | 99 | 99 | 99 | | | | |
| | 14 | | | | | | | | | | | | | | | | | | | 99 | 99 | 99 | 99 | | | | |
| | 15 | | | | | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| | 16 | | | | | | | | | | | | | | | | | | | 99 | 99 | 99 | 99 | | | | |
| | 17 | | | | | | | | | | | | | | | | | | | 95 | | | | | | | |
| 5 | 1 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 2 | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | | | |
| | 3 | 95 | | 95 | 95 | 95 | 95 | | | 99 | 95 | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | | | 95 | 95 | 95 | 95 | 95 | 95 |
| | 6 | | | | | | | | | | 95 | | | | | | | | | | | | | | | | |
| | 7 | 95 | 95 | 95 | 95 | 95 | 95 | | | 99 | 99 | | | 95 | 95 | | | | | 95 | 95 | 99 | 99 | | | | |
| | 8 | | | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 9 | | | | | | | | | 99 | 95 | | | | | | | | | | | | | | | | |
| | 10 | | 95 | | | | | | | 95 | 95 | | | 95 | 95 | 99 | 99 | 95 | 95 | | | 99 | 99 | | | | |
| | 11 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 99 | 99 | | | 95 | 95 | 99 | 99 | 95 | 95 | | | | | | | | |
| | 12 | 95 | 95 | 95 | 95 | 95 | 95 | 99 | 99 | 99 | 99 | | | 99 | 99 | 99 | 99 | 99 | 99 | | | | | | | | |
| | 13 | 99 | 95 | 99 | 99 | 99 | 99 | 99 | 95 | 99 | 99 | | | 99 | 99 | | | 95 | 95 | | | | | | | | |
| | 15 | 95 | | 95 | 95 | 95 | 95 | 95 | 95 | 99 | 99 | | | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | | | |
| | 16 | 95 | | 95 | 95 | | 95 | 99 | 99 | 99 | 99 | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | |
| | 17 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | 95 | 95 | 95 | 95 |
| | 18 | | | | | | | 99 | 99 | | | | | | | | | | | | | | | | | | |
| | 19 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | 99 | 99 | 99 | 99 | 99 | 99 | | | | | | | | |
| | 20 | | | | | | | 99 | 95 | | | | | | | 95 | 95 | | | | | | | | | | |
| | 21 | | | 95 | 95 | | | | | | | | | | | | | | | | | | | | | | |
| | 23 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | 99 | 99 | | | 95 | 95 | | | | | | | | |

TABLE XXIII - continued

Discriminant runs and equations

| Instar | Individual | 16 vars | 9 vars | 1 a | 1 a | 1 b | 1 b | 2 a | 2 a | 2 b | 2 b | 3 a | 3 a | 3 b | 3 b | 4 a | 4 a | 4 b | 4 b | 5 a | 5 a | 5 b | 5 b | 6 a | 6 a | 6 b | 6 b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 6 | 1 | 95 | 99 | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 |
| | 2 | | 95 | | | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | | | | | | | | | 99 | 99 | 99 | 99 |
| | 3 | | | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 4 | 95 | | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | 95 | | 95 | 95 | | | | | | | | |
| | 5 | | 95 | | | | | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | | | | | | | | | 99 | 99 | 99 | 99 |
| | 6 | | | | | 95 | 95 | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 7 | | | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 8 | | | | | | | 99 | 99 | 99 | 95 | | | | | | | | | | | 95 | 95 | | | | |
| | 9 | | | | | 95 | 95 | 99 | 99 | 99 | 99 | | | | | | | | | | | 95 | | | | | |
| | 10 | | | | | | | | | | | | | | | | | | | 99 | 99 | 99 | 99 | | | | |
| | 11 | | | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 12 | | | | | | | 99 | 99 | | | | | | | | | | | | | 95 | 95 | | | | |
| | 14 | 95 | 95 | 95 | 95 | 95 | 99 | 95 | 99 | 99 | 99 | 95 | 95 | 95 | 95 | | | | | | | | | 95 | 95 | 95 | |
| | 15 | | | | | | | 95 | 95 | 95 | 95 | | | | | | | | | | | | | | | | |
| | 16 | | | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 17 | | | | | 95 | 95 | 99 | 99 | | | | | | | | | | | 99 | 99 | 99 | 99 | | | | |
| | 18 | | | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | |
| 7 | 1 | 99 | 99 | 95 | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | | | | 99 | 99 | 99 | 99 |
| | 2 | 95 | 99 | | | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | | | 99 | 99 | 99 | 99 |
| | 3 | 99 | 95 | 95 | 95 | 95 | 99 | | | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | 99 | 99 | 99 | 99 |
| | 4 | 95 | 95 | 95 | 95 | 95 | 99 | | | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 |
| | 5 | 99 | 95 | 95 | 95 | 99 | 99 | | | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 |
| | 6 | | | | | | 95 | | | 95 | | 95 | 95 | | | 95 | 95 | 95 | 95 | | | | | | | | |
| | 7 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 99 | 99 | 95 | 95 | | | | |
| | 8 | 99 | 99 | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 |
| | 9 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | |
| | 10 | 95 | 95 | 95 | 95 | 99 | 99 | 95 | 95 | 99 | 99 | 95 | 95 | | | 95 | 95 | 95 | 95 | 95 | 95 | | | | | | |
| | 11 | 99 | 95 | 99 | 99 | 99 | 99 | | | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 |
| | 12 | 99 | | 95 | 99 | 99 | 99 | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | 95 | 95 | | | | |
| | 13 | | 95 | | | | | 99 | 99 | | | | | | | | | | | 95 | 95 | 99 | 99 | | | | |
| | 14 | | | | | | | | | | | | | | | | | | | | | 95 | 95 | | | | |
| | 15 | 95 | | 95 | 95 | 95 | 95 | | | 99 | 95 | | | | | | | | | | | | | | | | |
| | 16 | | | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | |
| | 17 | | | | | | | | | 95 | | | | | | | | | | | | | | | | | |
| | 18 | 99 | 95 | 99 | 99 | 99 | 99 | 95 | 95 | 99 | 99 | 95 | 95 | | | 95 | 95 | 95 | 95 | | | | | | | | |
| | 19 | 95 | | 95 | 95 | 99 | 99 | | | | | | | | | | | | | 95 | 95 | | | | | | |
| | 20 | | | | | | | | | 99 | 99 | | | | | | | | | | | | | | | | |
| | 21 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 22 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 95 | 95 | 99 | 99 | 99 | 99 |
| | 23 | | | | | | | 95 | | | | | | | | | | | | | | | | | | | |
| 8 | 1 | | | | | | | | | | | | | | | | | | | | | 99 | 99 | 95 | 95 | 95 | 95 |
| | 2 | 95 | 95 | 95 | 95 | 95 | 95 | | | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | 95 | 95 | | | | |
| | 3 | | 99 | | | | | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 4 | 95 | 99 | | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 5 | | | | | | | | | | | 95 | 95 | | | | | | | | | 95 | 95 | 99 | 99 | 99 | 99 |
| | 6 | | | | | | | | | | | 95 | 95 | 95 | 95 | | | | | | | 99 | 99 | 95 | 95 | 95 | 95 |
| | 7 | | | | | | | | | | | 95 | 95 | 99 | 95 | 95 | 95 | 95 | 95 | 99 | 95 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 9 | | | | | | | 99 | 99 | 99 | 99 | 95 | 95 | 95 | 95 | | | | | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 10 | | | | | | | | | | | | | | | | | | | | | 95 | 95 | | | | |
| | 11 | | | | | | | 99 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | | 95 | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 12 | | 99 | | | | | 95 | 95 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | | | | | 99 | 99 | 99 | 99 |
| | 13 | | 95 | | | | | | | | | 95 | 95 | | | 95 | 95 | 95 | 95 | | | 95 | 95 | 95 | 95 | 95 | 95 |
| | 14 | | | | | | | | | | | | | 95 | 95 | | | 95 | 95 | 95 | 95 | | | 95 | 95 | 95 | 95 |
| | 15 | | | | | | | 99 | 99 | 99 | 99 | | | | | | | | | 99 | 99 | 99 | 99 | | | | |
| | 16 | | 99 | | | | | 99 | 99 | 99 | 99 | | | | | | | | | 95 | 95 | 95 | 95 | | | | |
| | 18 | | | | | | | 99 | 99 | 99 | 99 | | 95 | 95 | 95 | | | | | 99 | 99 | 99 | 99 | 95 | 95 | 95 | 95 |
| 9 | 1 | 95 | 95 | | | 95 | 95 | | | | | | | 95 | 95 | | | 99 | 99 | | | 99 | 99 | | | 95 | 95 |
| | 2 | | | | | | | | | | | | | | | | | | | | | 95 | 95 | | | | |
| | 3 | | | | | | | | | | | | | | | | | | | | | 95 | 95 | | | | |
| | 4 | | | | | | | | | 99 | 99 | | | 95 | 95 | | | 99 | 99 | | | 99 | 99 | | | 95 | 95 |
| | 5 | | | | | | | | | | | | | | | | | | | | | 95 | 95 | | | | |
| | 6 | | | | | | | | | 95 | | | | 99 | 99 | | | 99 | 99 | | | 99 | 99 | | | 99 | 99 |
| | 7 | | | | | | | | | 99 | 99 | | | | | | | | | | | 99 | 99 | | | | |
| | 8 | 95 | 95 | | | 99 | 99 | | | | | | | 99 | 99 | | | 99 | 99 | | | | | | | 99 | 99 |
| | 9 | | | | | | | | | 99 | 95 | | | | | | | | | | | 95 | 95 | | | | |
| | 10 | | | | | | | | | 95 | 95 | | | 95 | 95 | | | 95 | 95 | | | 99 | 99 | | | 95 | 95 |
| | 11 | | | | | | | | | 99 | 99 | | | 95 | 95 | | | 95 | 95 | | | 99 | 99 | | | 99 | 99 |
| | 12 | 99 | 99 | | | 99 | 99 | | | | | | | 99 | 99 | | | 99 | 99 | | | 99 | 99 | | | 99 | 99 |
| | 13 | | | | | | | | | 99 | 99 | | | | | | | | | | | 99 | 99 | | | | |
| | 14 | | | | | | | | | 99 | 99 | | | 95 | 95 | | | | 95 | | | 99 | 99 | | | 99 | 99 |

TABLE XXIV

Individuals of the 26-9-71 test set misclassified by the 3-4-71 test set of discriminant function equations, and the instar these individuals were misclassifed to. An empty space indicates an individual was correctly assigned to instar

| Instar | Individual | Discriminant runs 16 vars | 9 vars | 1 a | 1 b | 2 a | 2 b | 3 a | 3 b | 4 a | 4 b | 5 a | 5 b | 6 a | 6 b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 12 | | | 6 | | | | | | | | | | | |
| 6 | 12 | | | | | | 7 | | | | | | | | |
| | 13 | | | | | | 7 | | | | | | | | |
| | 14 | | 7 | | | | 7 | | 7 | | | | | | 7 |
| 7 | 13 | 6 | | | 6 | | | | | | | | | | |
| | 15 | | | 8 | | | | | | | | 8 | | | |

**TABLE XXV**

**Individuals of the 26-9-71 test set whose discriminant values were outside the confidence limits of the 3-4-71 test set of discriminant function equations. 95 = 95%, 99 = 99%; underlining indicates a discriminant value outside the confidence limit of the lower tail of a sample distribution**

| Instar | Individual | 16 vars | 9 vars | Discriminant runs and equations | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | | | | 2 | | | | 3 | | | | 4 | | | | 5 | | | | 6 | | | |
| | | | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | | a | | b | |
| | | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 1 | 11 | | | 95 | 95 | | | | | | | | | | | | | | | | | | | | | | |
| | 13 | | | 95 | 95 | | | | | | | | | | | | | | | | | | | | | | |
| | 14 | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | | | |
| | 15 | | | 95 | 95 | | | | | | | | | | | | | | | | | | | | | | |
| 2 | 11 | | 95 | | | | | | | | | 99 | 99 | | | 95 | 99 | 99 | 95 | 99 | | | | 95 | 95 | | |
| | 12 | | | | | | | | | | | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | 95 | | | | 95 | 95 | 95 | 95 |
| | 13 | | 95 | | | | | | | | | | | | | | | | | | | | | | | | |
| | 15 | | | | | | | | | | | | | | | | | | | 99 | 99 | 95 | 95 | | | | |
| 3 | 11 | | | | | | | | | 95 | 99 | | | 95 | 95 | | | | | | | | | 95 | 95 | 95 | 95 |
| | 14 | | | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | |
| | 15 | | | | | | | | | 99 | 99 | 95 | | 99 | 99 | | | 95 | 95 | | | | | 99 | 99 | 99 | 99 |
| 5 | 11 | | | | | | | 99 | 99 | 95 | | | | | | | | | | | | | | | | | |
| | 12 | | 95 | | | | | 99 | 99 | 99 | 95 | | | | | | | | | | | | | 95 | 95 | 95 | 95 |
| | 15 | | | | | | | | | 99 | 99 | | | | | | | | | | | | | | | | |
| 6 | 12 | | | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 13 | | | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 14 | | 95 | | | | | 99 | 99 | 99 | 99 | 95 | 95 | | | | | | | 95 | | | | 95 | 95 | 95 | 95 |
| | 15 | | | | | | | 95 | 95 | 99 | 99 | | | | | | | | | | | | | | | | |
| 7 | 11 | | | | | | | 95 | 95 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 12 | | | | | | | 95 | 95 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 13 | | | | | 95 | 95 | 95 | | | | | | | | | | | | 95 | 95 | | | | | | |
| | 14 | | 95 | | | | | 99 | 99 | 99 | 99 | | | | | | | | | | | | | | | | |
| | 15 | | 99 | | | | | 99 | 99 | 99 | 99 | | | | | | | | | 99 | 99 | 99 | 99 | | | | |
| 8 | 11 | | | | | | | | | | | | | | | | | | | 99 | 99 | | | | | | |
| | 12 | | 99 | | | | | 99 | 99 | 99 | 99 | | | 95 | 95 | | | | | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| | 13 | | 95 | | | | | | 95 | 95 | 95 | | | | | | | | | 95 | 95 | | 95 | | | | |
| | 14 | | 95 | | | | | | | | | | | | | | | | | | | | | 95 | 95 | 95 | 95 |
| 9 | 11 | 95 | 95 | | | 95 | 95 | | | 95 | 95 | | | 95 | 95 | | | | | | | | | | | | |
| | 12 | | 95 | | | | | | | 95 | 95 | | | 95 | 95 | | | 99 | 99 | | | | | | | 95 | 95 |
| | 13 | | | | | | | | | 95 | 95 | | | | | | | | | | | | | | | | |
| | 14 | | 99 | | | | | | | 99 | 99 | | | | | | | | | | | | | | | | |

may have had a discriminant value which placed it outside the confidence limits of the set 2 sample discriminant values.

Summarizing, a new standardization set of discriminant equations calculated from a test set of data demonstrated that they gave optimal separation of the instars of this data set, but that their predictive power was lower for individuals of other data sets. As well, the predictive power of the set 2 equations was lower than the data set 1 standardization equations because the data on which the set 2 equations were based were less variable and the resulting discriminant values had narrower confidence intervals.

TABLE XXVI

Effect of lumping instars on Generalized Mahalanobis $D^2$ values, standardization set of A. tillyardianum data, using instars 4, 5, 6 and 7

| Comparison between instars | $D^2$ values for selections | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 4 -5 | 26 428 | 215 | 192 | 149 | 93 | 191 |
| 5 - 6 | 19 057 | 154 | 132 | 131 | 60 | 102 |
| 6 - 7 | 40 377 | 213 | 146 | 146 | 84 | 105 |
| 4 & 5 - 6 & 7 | 336 403 | 319 | 269 | 268 | 212 | 242 |

Effect of lumping instars on Generalized Mahalanobis $D^2$ values

As in the case of t-test values discussed previously (p.93 ), Generalized Mahalanobis $D^2$ values obtained when instars were lumped were larger than those obtained when instars were correctly separated (Table XXVI). Thus, these values cannot be interpreted as indicating whether instars have been lumped or not -- they indicate only the magnitude of distances between centroids of groups.

Fisher's linear discriminant function

This type of analysis was developed by Fisher (1936) to discriminate between two species of Iris, and subsequently has been widely used in biological, medical, psychological and anthropological studies (Dupraw, 1965; Crichton, 1966; Ciesielska & Kupść, 1968; Ouellette & Qadri, 1968; Choi & Trotter, 1970; Norris & Barkham, 1970; Amenta & Harkins, 1971; Stilmant et al., 1971, and others). The theory and calculations involved in using Fisher's linear discriminant function (= canonical variates analysis) are well documented by Cooley & Lohnes (1962, 1971), and Blackith & Reyment (1971).

In this study, programs DISCRIM, RSPACE and CLASSIF of Cooley & Lohnes (1962) were used to form the discriminant function equations, and to classify all individuals of the three data sets into instars according to these discriminant equations. A subset of 9 variables of the standardization set (numbers 2, 3, 4, 5, 7, 8, 9, 10, and 11) was the only data subset used.

It was found that 99.4% of the total discriminating power of the discriminant functions was contained in the first three discriminant function equations. Wilks' lambda criterion indicated that there was very good separation between the nine instar groups ($\Lambda = 0.0013$; $d.f._1 = 72$, $d.f._2 = 841$, $F = 23.1$, $p << 0.001$). The centroids of the nine instars of A. tillyardianum have been projected onto the three dimensional reduced space of the first three discriminant function equations in fig. 5 , and it can be seen that most of the discriminating power is contained in the first discriminant function. This is a result of the gradual increase in size from instar to instar of all variables used in the analysis.

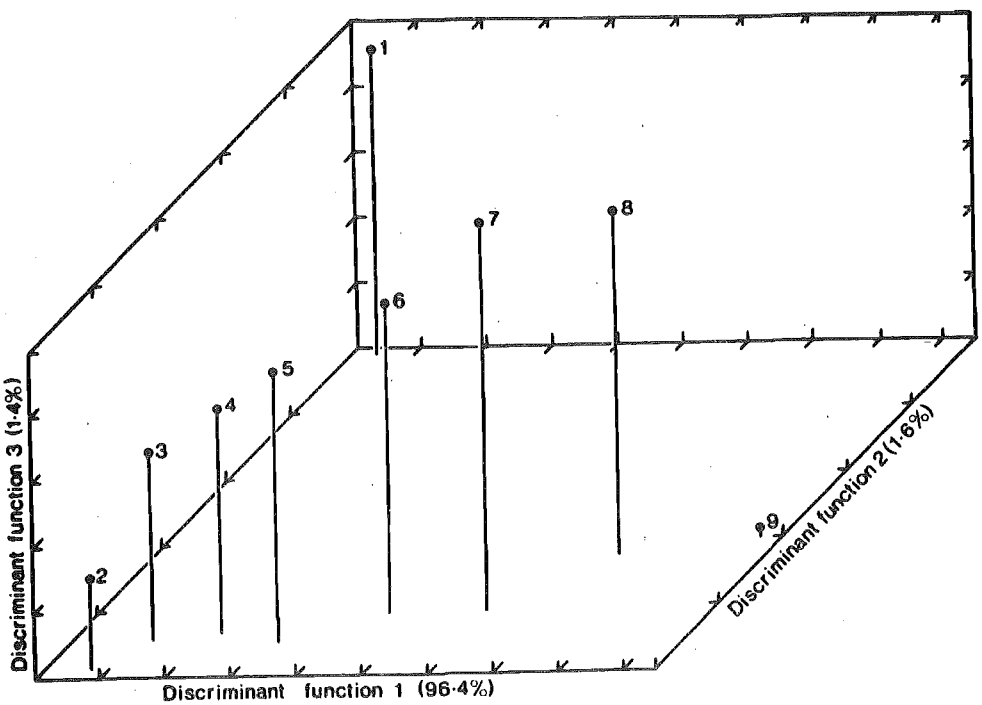



**FIG. 5.** 3-dimensional projection of the centroids of the nine instars of the standardization set of *A. tillyardianum* larvae in the reduced space of the first three discriminant functions. (The scale marks on the axes refer to discriminant values, which are artificial values formed from the original measurements).

TABLE XXVII

Instars to which individuals were assigned using Fisher's linear discriminant function , equations calculated from standardization set

Standardization set

| Instar | Instar 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 12 | | | | | | | | | |
| 2 | | 7 | | | | | | | | |
| 3 | | | 19 | | | | | | | |
| 4 | | | | 18 | 1 | | | | | (3 out of 154 misclassified) |
| 5 | | | | 1 | 22 | 1 | | | | |
| 6 | | | | | 1 | 18 | | | | |
| 7 | | | | | | | 24 | 1 | | |
| 8 | | | | | | | 1 | 17 | | |
| 9 | | | | | | | | | 14 | |

Test set 3-4-71

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 9 | 1 | | | | | | | | |
| 2 | 1 | 10 | | | | | | | | |
| 3 | | | 10 | | | | | | | (11 out of 90 misclassified) |
| 4 | | | | 8 | 2 | | | | | |
| 5 | | | | 2 | 10 | 2 | | | | |
| 6 | | | | | 2 | 8 | 1 | | | |
| 7 | | | | | | 1 | 9 | 4 | | |
| 8 | | | | | | | 4 | 6 | 1 | |
| 9 | | | | | | | | 1 | 9 | |

TABLE XXVII-- continued

Test set 26-9-71

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4 | 1 | | | | | | | |
| 2 | 1 | 5 | | | | | | | |
| 3 | | | 5 | | | | | | |
| 4 | | | | 4 | 1 | | | | |
| 5 | | | | 1 | 5 | 1 | | | |
| 6 | | | | | 1 | 4 | 1 | | |
| 7 | | | | | | 1 | 4 | 2 | |
| 8 | | | | | | | 2 | 3 | |
| 9 | | | | | | | | | 5 |

(6 out of 45 misclassified)

Efficiency of classification

Most individuals of the standardization set were correctly classified to instar in the analysis (Table XXVII), and in fact fewer were misclassified than with the quadratic discriminant function equations (3 misclassified as opposed to 8). Similarly, Fisher's linear discriminant function was more efficient than the quadratic equations in classifying individuals of the 3-4-71 test set to instar (11 as opposed to 16), but was less efficient in classifying the 26-9-71 test set (6 as opposed to 3).

It was particularly interesting to note that most of the misclassified individuals were of instars 4 to 8, as it is individuals of these instars that are at times difficult to separate by morphological characters. Using quadratic discriminant functions, however, the trend was for instars 6 to 9 of the test sets to be misclassified rather than the smaller instars -- seasonal differences in size appears to be the best explanantion for the misclassifications. It remains a puzzle though as to <u>why</u> there should be this misclassification difference between the two methods of discriminant function analysis.

Seasonal effects on instars

No variation in instar number was found during the period of investigation; there were, however, some statistically significant differences in size of structures of instars between collection dates as shown by t-tests (Table XXVIII). Instars 6 to 9 of the 3-4-71 test set were, in general, smaller than corresponding instars of the standardization set and 26-9-71 test set, whereas the first 4 instars were slightly larger (fig. 3); instar 5 larvae did not differ significantly in size. Although in the above cases size differences were statistically significant, visually the differences in size of an instar were not apparent, and larvae from a collection could be sorted into instars by general size without difficulty. In the discriminant function analyses, these small size differences between larvae of the same instar from different collection dates were compounded, and the effect was to make the instars of the different collections appear more different than they really were (p. 115). Thus, although larvae of an instar from different dates were statistically different and separable according to size, these differences could not be related to any morphological or biological differences.

Summarizing, no seasonal change in instar number of A. tillyardianum was found, and conclusions based on the data presented should be applicable to future collections of larvae.

TABLE XXVIII

T-tests between the standardization set and two test sets of larvae for the nine instars of A. tillyardianum for ten variables to test for seasonal differences

| Instar | Data sets | Variables | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 11 |
| 1 | 1-2 | ** | | | | | | - | - | *** | |
| | 1-3 | ** | * | | | | * | - | - | | |
| | 2-3 | | | | | | | - | - | | |
| 2 | 1-2 | | | | | | * | | - | * | |
| | 1-3 | ** | | | ** | | | | - | | |
| | 2-3 | ** | | | | | * | | - | | |
| 3 | 1-2 | * | *** | ** | * | ** | | | | | |
| | 1-3 | * | * | ** | *** | ** | | | ** | | |
| | 2-3 | | | | | | | | * | | * |
| 4 | 1-2 | ** | *** | * | * | | | | * | | |
| | 1-3 | | *** | *** | *** | | | | | | |
| | 2-3 | | | | | | | | * | | |
| 5 | 1-2 | | | | | | | | * | | |
| | 1-3 | ** | | | | | | | | * | |
| | 2-3 | | | | | | | | | | |
| 6 | 1-2 | | | * | * | | * | | ** | ** | ** |
| | 1-3 | | * | | | | | | | ** | |
| | 2-3 | | * | * | * | | | | | | ** |
| 7 | 1-2 | ** | | ** | ** | *** | ** | *** | ** | *** | ** |
| | 1-3 | | | | | | * | * | | *** | |
| | 2-3 | ** | | | | | | | * | | * |
| 8 | 1-2 | | * | * | *** | *** | * | ** | ** | * | |
| | 1-3 | | * | | | * | | * | | ** | |
| | 2-3 | | | ** | *** | | | | | | |
| 9 | 1-2 | | | ** | ** | *** | *** | * | *** | * | |
| | 1-3 | * | * | | | ** | | * | | | |
| | 2-3 | ** | * | ** | * | | * | | | | |

Data sets

1 = 29-9-70 & 18-10-70

2 = 3-4-71

3 = 26-9-71

Significance levels

* = 0.01-0.05

** = 0.001-0.01

*** = < 0.001

\- = no comparison

## Discussion

### Evaluation of previous simuliid instar determination studies

Although several detailed instar determination studies have been made over the last sixteen years (Table I, A), each has suffered from at least one of four limitations.

1. Larvae used often came from mixed simuliid species streams, and it is problematical whether all the young larvae were of the species investigated. It is imperative to ensure that larvae used come from a single species stream, or from rearing from egg to adult.

2. Generally the number of early stage larvae used was small. This resulted in early instars tending to be lumped because of the apparently insufficient separation caused by the low numbers of specimens.

3. The larvae often were collected at different times of the year or in different years. Therefore, measurements could either reflect the seasonal variability of the instars for univoltine species, or, when measurements for instars at different dates were lumped, reflect the variability between generations for a multivoltine species.

4. The magnification used for measuring is usually not mentioned. An increase in variance of a measurement for an instar can sometimes be related to a change in the magnification used for measuring, and this lessens the demarcation between instars. If a structure is measured at too low a magnification, there will be little separation between the early instars, resulting in their lumping.

The following critical evaluation of previous instar determination studies illustrates many problems associated with instar determination in simuliids; in particular, those requiring an investigator to work with field collected larvae from mixed-species populations, and also with species that are readily available for only part of a year. Familiarity with the limitations and problems of instar determination means that the reliability of these studies can be assessed in light of my A. tillyardianum study, and that some of these results can be re-interpreted to give a higher number of larval instars.

Terteryan (1957)

After detailed morphological and biometrical analyses, Terteryan concluded that Simulium (Odagmia) kiritshenkoi Rubzov and Simulium (Wilhelmia) paraequinum transcaucasicum (Rubzov) (presumably this subspecies according to Crosskey (1967)) had six larval instars. Specimens for this study were collected at all times of the year, but in particular in the spring-summer and summer-autumn transition periods. Presumably the specimens came from mixed-species populations, but this is not stated. Larvae were placed into groups by eye according to their head widths and the head width of a specimen from each group was measured. The remaining specimens were allowed to moult, and were then measured. After the number of instars was established as six, specimens of each instar were measured and each instar was morphologically characterized.

As well as measuring sclerotized structures of the larval head capsule, Terteryan measured decapitated body length and greatest thorax width. The mean, standard error of the mean and standard deviation were presented for each measurement of an instar, but no statistical tests were undertaken to test the significance of differences between means of different instars. Since sample sizes for instars were not stated, it is not possible to test the differences statistically. However, inspection of the data for both species suggests that there are probably significant differences between mean values for each measurement in the groups he considers to be instars.

TABLE XXIX

Widths of larval head capsules measured between the eye spots of Simulium (Wilhelmia) paraequinum transcaucasicum, adapted from Terteryan (1957: Table 3). Figures in parentheses are the presumed units on the measuring scale used by Terteryan

| | Width of head capsule between eye spots (mm) | | | | | |
|---|---|---|---|---|---|---|
| | "already moulted" | | | "just moulted" | | |
| Instar | Min. | Max. | Range | Min. | Max. | Range |
| 1 | 0.093 (5) | 0.113 (6) | 0.020 | - | - | - |
| 2 | 0.131 (7) | 0.188 (10) | 0.057 | 0.093 (5) | 0.150 (8) | 0.057 |
| 3 | 0.226 (12) | 0.301 (16) | 0.075 | 0.168 (9) | 0.281 (15) | 0.113 |
| 4 | 0.339 (18) | 0.414 (22) | 0.075 | 0.319 (17) | 0.414 (22) | 0.095 |
| 5 | 0.452 (24) | 0.527 (28) | 0.075 | 0.432 (23) | 0.508 (27) | 0.076 |
| 6 | 0.565 (30) | 0.770 (41) | 0.205 | 0.535 (28.5) | 0.603 (32) | 0.068 |

Terteryan found that the best measurement for separating instars was the width of the head capsule after moulting, and showed that for S. p. transcaucasicum the absolute minimum and maximum values of this measurement were lower for those specimens "just moulted" into an instar than those specimens "already moulted" to that instar. However, what he did not state was that the ranges for both sets of measurements were similar, and that in fact for the supposedly less variable set of "just moulted" early instars, ranges were greater than the corresponding ranges for early instars of the "already moulted" set (Table XXIX).

Some of his values suggest lumping of instars. In the "just moulted" set of S. p. transcaucasicum head widths, which were the "[most valuable confirmation of the number of stages]", ranges of instars 3 and 4 are greater than those of the "already moulted" set. Further, the range of instar 3 is greater than the range of any other instar of the "just moulted" set (Table XXIX). Part of the reason for the large ranges of early instars may be accounted for by the measuring scale Terteryan is presumed to have used. All minimum and maximum values of the head capsule measurements (for both species) are consistent with a scale of one division equalling 18.7 to 18.8 µm being used. On such a scale, the minimum value given for instar 1 is equivalent to 5 units, and the maximum value given for instar 6 is equivalent to 41 units (Table XXIX). Measurements for instars 1 to 4 lie within the range of 5 to 22 units, a scale of only 17 units. It would be difficult to separate early instars on such a restricted scale, and the tendency would be to lump instars if there were no distinct gaps in the frequency distribution of a measurement. The wide ranges of the early instars suggest that Terteryan did in fact lump some of his early instars.

However, it could be that the instars of the species studied were of different sizes in the different seasons. Any clear demarcation between instars within a season may have been masked when specimens from all seasons were combined. It is doubtful, though, if all the variability in the early instars can be attributed to this source of errror.

The illustrations of each instar showing size of the head capsule and antennae are misleading as they are not drawn to scale relative to each other according to the mean measurements given by Terteryan. Grenier & Feraud (1960), on the basis of these illustrations, suggested that another instar could be interpolated in the large gap between instars 4 and 5. But when the structures are drawn to scale relative to each other using the mean measurements, no apparent gaps between instars exist. On the other hand, if the illustrations are of individuals of different instars that have been drawn to scale relative to each other then Grenier & Feraud's supposition is not altogether unfounded, and Terteryan may have overlooked (or lumped) one or several instars. A closer examination of the earlier stages probably would be rewarding.

Grenier & Feraud (1960)

Grenier & Feraud's study on the common African onchocerciasis-carrying species Simulium (Edwardsellum) damnosum Theobald demonstrated the presence of seven larval instars. Separation from instar 4 onwards was assisted by the gradually increasing number of scales on the thoracic and abdominal cuticle, a morphological feature characteristic of the subgenus Edwardsellum.

Two hundred and seventy-nine larvae were collected at the same time from the one attachment site in a river. Larvae were from a mixed-species population, but specific identification was possible though checking the characteristic shape of the submental cleft. A problem with basing conclusions on the number of instars for a species on one collection is that some instars may not be present at that particular sampling time. In a fast growing species such as S. damnosum with a larval life of about a week (Wanson & Henrard, 1945; Kuzoe, 1969) it is quite probable that an instar may not be present. This opinion is reinforced by the fact that only 18% of specimens were of the first three instars.

Instar groupings were provisionally made according to body length and head capsule size of larvae. The first three and final two instars could be defined morphologically by previously known characters (Terteryan, 1957), and the amount of covering of the cuticle by scales provided morphological evidence for two intermediate instars.

The best measurement to show the presence of seven instars was that of mandible length (Grenier & Feraud, 1960: Figure 1): however, the division into instars based on mandible length is not entirely convincing.

The histogram peaks are not clear-cut, and other interpretations of where the divisions should lie are possible. For example, an instar could be interpolated between 190 and 215 μm, splitting instar 6 into two. Similarly, it is possible to interpret instar 4 as two instars. Perhaps closer examination would reveal morphological features which would support such divisions.

The statistical section of the study done by Itard and Le Berre purported to show statistically significant differences between mandible lengths for Grenier & Feraud's instars 4, 5, and 6 by using F-ratio tests. However, all that was shown was that the variances of these instar groupings were heterogeneous: at no stage were statistically significant differences between the means of the instar groupings demonstrated. Using an adjusted t-test (Sokal & Rohlf, 1969) it is possible to show that there are statistically significant differences between the means, but such a test is meaningless in the present context when the division into the instar groupings is dubious (see p. 89 ).

Although an apparently reliable study, the limitations outlined above suggest that the results obtained are not definitive, and therefore should be regarded with some caution, especially when considering the less well characterized intermediate instars.

Yakuba (1960)

Based on the supposition that simuliids had the same number of instars as mosquitoes, Yakuba apparently divided up his data to show that Simulium (Wilhelmia) equinum (Linnaeus) and Simulium (Simulium) galeratum Edwards had four instars! Morphological evidence was not considered.

The time of the year the larvae were collected is not mentioned, nor is it indicated if they were from mixed-species populations. It is stated, though, that some of the larvae used were those kept alive in the laboratory. The head capsule length and width were measured for about 7 000 larvae, but data for only 350 S. equinum and 600 S. galeratum were analyzed. Of these larvae, fewer than 20% were in Yakuba's instar groupings 1 and 2.

In forming the four instar groupings from the measurements, Yakuba appears to have arbitrarily divided the continuous scales and disregarded any morphological evidence. It can be shown with t-tests that statistically there are highly significant differences between the instar groupings, but such statistical tests are meaningless here when the groupings are unsound (See p. 89 ).

All minimum and maximum values given for head capsule measurements (for both species) are consistent with a scale of one division equal to 12.5 µm being used. There would be problems in separating early instars on such a scale -- these have been discussed under Terteryan (1957).

It must be concluded that Yakuba has failed to give convincing evidence that the two species he studied had four larval instars, and the

means, standard deviations and standard errors of the means presented for the instar groupings should be regarded as being unreliable.

Grant (1961)

This study on Simulium (Odagmia) ornatum Meigen from England leaves much to be desired. Apart from mentioning that his "observations were made on thousands of living specimens in the streams of . . . Bristol", no indication is given of what time of the year the larvae were collected, whether all instars were present in his collections, or if S. ornatum was collected from mixed-species populations. Six instars were considered to be present and were separated on the basis of morphological features; instar 1 by the presence of an egg burster, and the remaining five instars by the appearance and relative growth of the imaginal buds plus increase in body length.

The measurements given for body length strongly suggests that they were guessed and not actually measured. There is no correspondence between the measurements presented by Grant and those given for S. ornatum from Scotland by Smart (1934). For example, Grant stated that instar 1 was about 2.0 mm long, yet according to Smart, a well-grown instar 3 was still only about 1.5 mm long. Grant also stated that instars 5 and 6 were both about 9.0 mm long but Smart found that the penultimate instar was about 5.0 mm long and the final instar was about 6.5 to 8.0 mm long.

On the basis of the information presented, Grant's attempt to determine the number of larval instars for S. ornatum should be regarded as being unreliable.

Harrod (1964)

Simulium (Odagmia) nitidifrons Edwards was thought to have six larval instars by Harrod. Width of the cephalic apotome (frons-clypeus) was the parameter used to separate the instars, and further characterization was possible using the morphological features of cephalic fan ray number and relative development of the imaginal buds.

Larvae for her study were laboratory reared from eggs. This overcame the problem of working with a mixed-species population, and also provided larvae that had been grown under known conditions. Although the number of larvae measured was not stated, indirect evidence from Hall & Harrod (1963) indicated that high numbers were used.

However, separation of the intermediate instars is somewhat suspect. The characterization of instar 4 by cephalic apotomewidth does not appear to be fully justified as the range for this measurement is greater for instar 4 than any other instar. Also, the number of cephalic fan rays in instar 4 is more variable than in other instars. These facts suggest that two instars were probably lumped by Harrod to give instar 4.

Kačanski (1968)

Morphological and biometrical evidence were used by Kačanski to show the presence of seven larval instars in a Yugoslavian population of Simulium (Odagmia) ornatum Meigen.

Larvae used were those of the first generation of the year. A series of 344 larvae, about 50 from each instar, were measured and morphologically characterized. For each of the measurements taken, the mean, standard error of the mean, standard deviation, and coefficient of variation were given. Separation of instars 1-3 was based on the number of antennal segments present, and instar 7 was characterized by the separation of the cervical sclerites from the postocciput and development of the respiratory histoblast. Morphological differences between instars 4, 5 and 6 were slight, and separation was based on the relative growth of the antennal segments and on the amount of sclerotization of the cervical sclerites.

In the first three instars, there is some overlap between minimum and maximum measurements amongst adjacent instars. However, since these instars were separated by antennal segment morphology, it is unlikely that this overlap would indicate lumping. The high coefficients of variation for the measurements of instars 4, 5 and 6 could indicate that the instars were incorrectly separated in this region, but it is possible that a change of measuring scale between instars was the cause of the high coefficients of variation (also see A. tillyardianum data, Tables III - V ).

However, the approach taken by Kaćanski, and the care with which the instars were separated, would make her study the best of previous studies.

Smith (unpublished 1969)

Smith considered that Prosimulium (Prosimulium) hirtipes (Fries) had eight larval instars, a conclusion based primarily upon width of the head capsule between the eye spots, and secondarily upon morphological characters.

Some 6 630 P. hirtipes larvae from different streams, years and sampling dates were measured by Smith. Larvae were collected from mixed-species streams, but P. hirtipes was "quite distinct from those of the other species present at all stages in their development". Head capsules were removed from the rest of the body before measuring head widths at x100; larvae were then discarded. The resulting histogram of head capsule widths was subjected to a polymodal frequency analysis. The eight instars are not well defined in this analysis -- it is possible that they would be better defined if the different years and sampling dates were analyzed separately.

To discover if there was a correspondence between measurements and morphological features of an instar, a further 60 specimens were examined in detail at a later date. It was found that instars 1, 2, 3, 4 and 8 could be characterized morphologically as well as biometrically, instars 5 and 7 were less well defined morphologically, whereas instar 6 could not be defined morphologically and evidence for its existence was only given by the polymodal frequency analysis.

It should be noted that Smith found only two first instar larvae, this was interpreted as indicating that the first instar was of short duration, as shown for a Canadian species of Prosimulium by Davies (1960).

Although Smith's conclusions are based on large numbers of larvae, separation of the instars is not clear-cut. It may have been more profitable to study fewer larvae in greater detail.

Johnson & Pengelly (1970)

According to Johnson & Pengelly, Simulium (Simulium) rugglesi Nicholson & Mickel had seven larval instars, and the instars defined using this measurement appeared to conform with Brooks' rule.

Over a one month period 580 larvae were collected from one sampling area, and the cephalic apotome width of these larvae was measured at x100. It appears as if the measuring scale used was 1 unit equivalent to about 14 µm, and that measurements were taken to the nearest 1/2 unit. Thus the seven instars were separated on a scale of 38 half-units, and, moreover, the first four instars by a scale of only 15 half-units. Although the final three instars are well defined, the first four instars are not well demarcated, probably as a consequence of the measuring scale used.

Morphological evidence for the separation of the final three instars was provided by the growth of the imaginal buds. The shape of the sub-mental cleft and the general body coloration were shown to change from instar to instar, but these changes were not sufficient to characterize each instar.

In the absence of evidence that early instars were separated on antennal morphology, separation of these instars must be regarded as tentative. However, the striking separation of the final three instars by cephalic apotome width should be regarded as being conclusive.

Application of A. tillyardianum results to field situation

The ultimate purpose of this detailed study on instar number in A. tillyardianum was to apply the results to a field study on population changes. This aim was achieved because of the relationship established between morphology and general size of structures of instar groups. It was possible to sort larvae into instars by overall general size, and then to check finer morphological characters of larvae to ensure they had been correctly assigned to instar. In the population study, the instar of up to 100 larvae $h^{-1}$ could be determined using a dissecting microscope and magnifications no greater than x40.

Although larvae had to be sorted into nine instar groups, hereby considerably increasing sample sorting time, the high number of instars proved to be of value to the study, as it assisted in separating overlapping generations of A. tillyardianum.

Significance of nine instars in A. tillyardianum

The presence of nine instars in A. tillyardianum raises the question, is nine instars typical for the genus Austrosimulium Tonnoir?

Confirmatory evidence for there being nine instars in other Austrosimulium is as yet not available. An Australian species Austrosimulium (Novaustrosimulium) bancrofti (Taylor) appears to have seven instars based upon head capsule widths (M. Colbo, pers. comm.). Examination of collections of other Austrosimulium species from South Island localities indicates they may have nine instars. However, in most localities, two or three species usually are found together, often on the same rock, and early instars of different species can not be separated.

Laboratory rearing from egg to adult provides the only certain method of obtaining all larval instars. To ensure that all stages are in fact collected in laboratory cultures, samples from the cultures should be taken at least daily; this means large numbers need to be reared. Although some simuliid species have been reared from egg to adult (Hartley, 1955; Doby, David & Rault, 1959; Hall & Harrod, 1963; Raybould, 1967), in most cases larger larvae only have been reared through to adults (Odintzov, 1960; Wood & Davies, 1966).

I was not successful in rearing A. tillyardianum beyond instar 7 from eggs. In my field sampling programme, however, I collected a complete series of newly moulted larvae, each larva with its head capsule of the preceding instar, and this confirmed the presence of 9 larval instars and the lack of any sexual dimorphism in large larvae.

The need now is for the instar number of species already studied to be re-examined, and for species of other genera to be studied, such as Cnephia Enderlein, Metacnephia Crosskey and Twinnia Stone & Jamnback. Also, larvae of one species need to be reared under different conditions as it is not known what effects environmental conditions have on instar number -- if they have any. There may be differences between species or genera, for example, based on whether they are from cold or warm water regions of the world, or whether a species is univoltine or multivoltine. Once such studies have been undertaken, it may be possible to establish relationships between simuliids based on larval instar number.

Summary

The nine larval instar groupings of Austrosimulium (Austrosimulium) tillyardianum Dumbleton constructed on the basis of morphological differences are shown to be biometrically discrete by using four methods of analyzing measurements.

Three sets of larvae were used in the study, a standardization set and two test sets collected at different times. Sixteen variables were recorded for each larva, ten were measurements, two referred to the number of teeth or hairs on structures, and four were character states of structures which served as dummy variables.

Placing individuals into instar groups according to frequency distributions of measurements is not very effective for A. tillyardianum as boundaries between instars are not clear-cut.

Brooks' rule (Dyar's rule) is shown to apply to A. tillyardianum measurements. A significant deviation from a straight line logarithmic progression is defined by a "growth ratio rule" to discover if instar categories have been lumped. Application of this rule to other simuliid studies suggests lumping of instars in several cases.

Student's t-tests between means of instar groups for all measurements of A. tillyardianum indicated significant differences between the groups. However, it is shown by haphazard division of a continuous measurement

into groups and by lumping instar groups, that significant differences between groups does not necessarily prove the validity of the groups.

The multivariate statistical method of multiple discriminant function analysis indicated that the A. tillyardianum instar groups were discrete, and the discriminant equations calculated could correctly assign 90-95% of individuals to their instar. Classification accuracy for a discriminant run was related to the subjective assessment of the reliability of the measurements in separating instars. Discriminant equations calculated from the standardization set of larvae were found to have good predictive power in assigning larvae of the test sets to instar. However, discriminant equations calculated from the less variable 3-4-71 test set were found to be less useful in assigning other larvae to instars.

A critical evaluation of previous simuliid instar determination studies shows the results of some can be re-interpreted to give a higher number of larval instars. It appears as if several species of Simulium may in fact have eight instars, although closer examination of more material of these species will be required to find out if the suggested divisions are justified.

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'. . . population ecology, apart from its exciting theoretical aspects, contains much that is best described as unadulterated drudgery.'

Park (1954) *Physiological Zoölogy* 27:186

Paper 3

# Population changes of *Austrosimulium tillyardianum* in an experimental channel of a New Zealand stream (Diptera : Simuliidae)

## INTRODUCTION

Simuliidae are found throughout the world where suitable riffle areas in streams and rivers exist for larvae. Females of many species are regarded as pests because of their bloodsucking habits (Fallis 1964), and a few species are important as vectors of parasites. In Africa and Central America some transmit filaroid nematodes which cause onchocerciasis and may result in blindness to man (Lewis 1953; Dalmat 1955; De León 1957; Roberts et al. 1967; Duke & Moore 1968; etc.), and other species in North America and Britain transmit Leucocytozoon spp. (Protozoa: Sporozoa) to birds (Fallis & Bennett 1962; 1966; Baker 1970; etc.). It is possible that some species may transmit viruses (Anderson et al. 1961), and it appears as if Austrosimulium (Austrosimulium) ungulatum Tonnoir from New Zealand could act as a mechanical vector of two arboviruses under favourable conditions (Austin 1967). Despite their medical and veterinary importance little is known about population changes in simuliids because of difficulties in studying the larval stages and separating species in mixed-species samples.

The purpose of this paper is to present information on population changes in a common, mainly non-manbiting, New Zealand simuliid Austrosimulium (Austrosimulium) tillyardianum Dumbleton in an experimental channel of a stream, and by doing so, to indicate the potential of studying population changes using an experimental situation located in a natural area.

A. tillyardianum was the only simuliid present in the Wainui Valley Stream (Crosby in press "a") and larvae were readily available throughout the year. Further, of the 19 other invertebrate species collected from riffle areas, only four were abundant (Crosby in prep.) thus reducing the amount of possible interspecific interactions such as competition for attachment sites. Weekly sampling over a seven month period when stream

temperatures were relatively uniform was undertaken. In this time nine cohorts were followed through their life histories. Although the percent standard errors of means for the life history stages were between 20-35% and did not attain the 10% accuracy suggested for studying population changes (Southwood 1966), this is the first time an attempt has been made to calculate such estimates for a simuliid species, and the results were sufficiently accurate to indicate major changes.

Previous studies undertaken on population changes in larval simuliids have been concerned mainly with determining the number of generations in a year and seasonal changes. Many have presented information on changes in total numbers in samples containing several species collected on different occasions (Zahar 1951; Sommerman et al. 1955; Wolfe & Peterson 1959; Carlsson 1962; Patrusheva 1962, 1963; Abdelnur 1968; Ladle et al. 1972) or changes in numbers of different species with time (Dalmat 1955; Cariaso 1962; Hájková-Hlisnikovská 1962; Phelps & DeFoliart 1964). In other studies, larvae collected have been placed into categories according to different criteria, for example, general size, body length, head capsule measurements or instars, and the results have been expressed as numbers or percentage compositions. General size classes expressed as numbers present were used by Živković (1955), Davies (1957, 1961) and Chutter (1972) to show population changes, and Wurmbach (1928) gave his results as percentages. Maitland & Penney (1967) presented changes in numbers according to millimetre body length categories, and both Obeng (1967) and Ladle et al. (1972) used percentages for the same measurement. Categories formed on the basis of head capsule widths, expressed as percentages, have been used by several authors (Davies & Smith 1958; Mackay 1969; Smith 1969; Rühm 1970b). Placing larvae into instar categories so as to trace population changes has not often been used. Percentage change in instar composition was determined by Baranov (1926) and

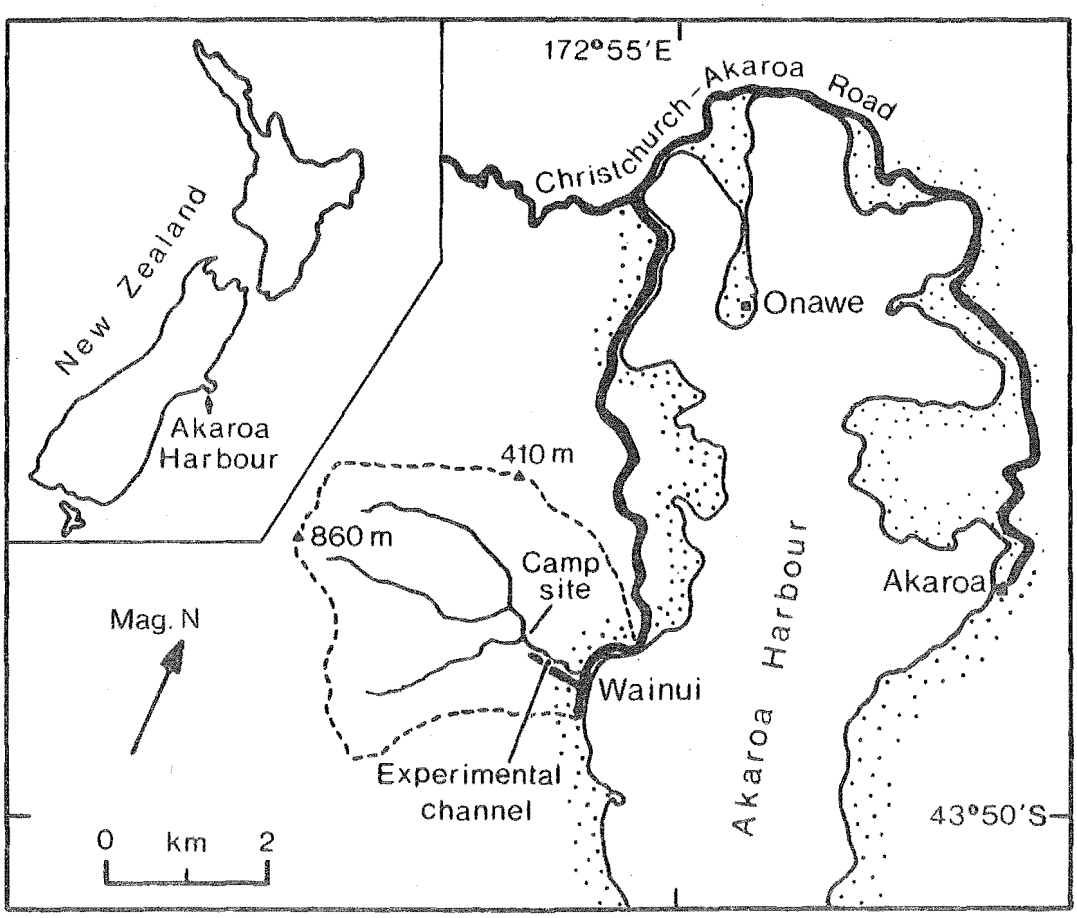


Fig. 1. Map of Akaroa Harbour area to show the location of Wainui Valley (limits indicated by dashed lines) and the position of the experimental channel. Inset, map of New Zealand.

Kačanski (1970) for the multivoltine species Simulium (Odagmia) ornatum Meigen, and for the univoltine species Prosimulium (Prosimulium) hirtipes (Fries) and Prosimulium (Prosimulium) arvernense Grenier by Smith (1969) and Halgoš (1972) respectively. The causes of population changes, or their extent, were not discussed. Kačanski (1970) also presented her data as mean numbers in each instar on each sampling date, but in doing this this lumped heterogeneous data from seven stations in the Žunovnica stream and only discussed seasonal changes and number of generations.

## STUDY AREA

The study area was located at Wainui Valley, Banks Peninsula, New Zealand (43°49'S, 172°54'E), a valley with an area of about 1 100 ha (Fig. 1). The valley rim is part of a basaltic lava dome of Pliocene age, and the lower slopes are covered with wind-blown loess (Harris & Harris 1939; Gage 1969). Soil near the stream is Wainui silt loam and soil of the general valley floor is Brough silt loam (Harris & Harris 1939). The original vegetation of the floor and slopes of Wainui Valley was forest and scrub but this was cleared and burnt-off between 1850 and 1880 and replaced with pasture grass (Petrie 1963). Now only remnant areas of native forest line and overhang the stream of the valley slopes, and a few trees overhang the stream along its lower course. Since A. tillyardianum is found characteristically in open, warm streams, it is probable that this vegetation change has enhanced conditions for the species in the stream.

The Wainui Valley Stream is a permanent stream which arises at about 600 m altitude and flows approximately 4.5 km before discharging into the west side of Akaroa Harbour. Two tributaries join the stream about 1.1 km and 1.5 km from the mouth. The stream is 2-3 m wide and 0.1-0.8 m deep

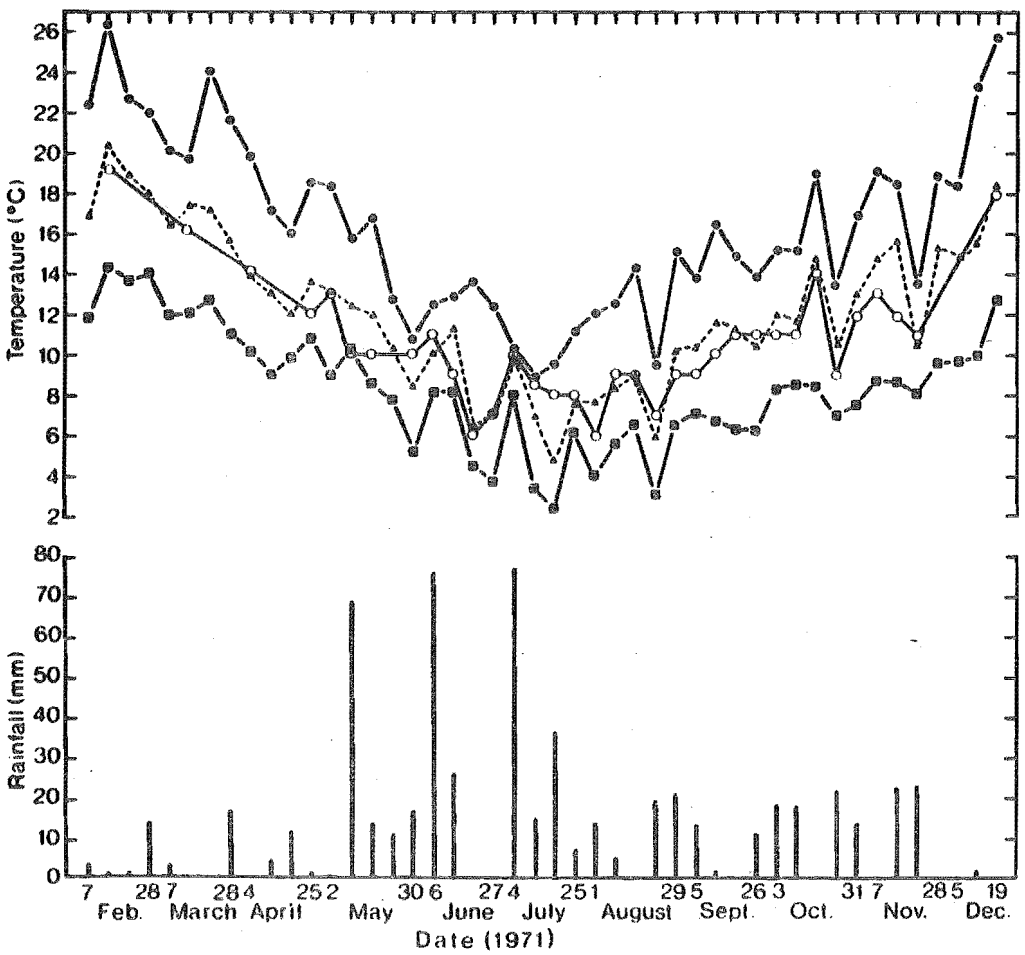


Fig. 2. Mean weekly temperatures and amount of rainfall recorded at Onawe, and stream temperature of the Wainui before, during and after the sampling period. ●, maximum temperature; ■, minimum temperature; ▲, temperature at 0900 h; O, spot stream temperature.

over its lower course. It consists of a series of riffles up to 10 m long separated by pools up to 30 m in length. The stream bed of the riffle areas is composed of stones varying in size from below 30 mm to over 1 m in diameter (Plate 2b), and the beds of the pools are mud covered with a few large stones. A. tillyardianum is found mainly in the lower 1.5 km of the stream, and is abundant only in riffle areas.

Wainui Valley is a popular weekend and summer holiday area, and there are holiday houses within 50 m of the stream (Plate 2b). There is also a Y.M.C.A. camp site by the stream about 1.1 km from the mouth (Fig. 1). Because of the risk of human interference, no recording instruments were left in the study area between sampling dates. Since interference was most likely to occur in weekends, sampling was undertaken on Sundays between 1400-1600 h New Zealand Standard Time so that if the experimental channel had been interfered with, the disturbance could be rectified probably within 24 h. Disney (1972) overcame problems due to human interference by use of ju-jus (principally semi-mummified monkey heads), but it is unlikely that such methods would have been successful in the present study!

Daily climatological observations are made at an official New Zealand Meteorological Service Station at Onawe, about 5 km north of Wainui Valley (Fig. 1). These observations were similar to the less detailed observations made at two other stations in the Akaroa Harbour area, and therefore were used as an indication of prevailing temperatures and rainfall in Wainui Valley (Fig. 2). The close relationship between mean weekly 0900 h temperatures at Onawe and stream temperatures at times of sampling ($r = 0.94$) meant that estimates of development time for cohorts, in terms of "accumulated degree-days above 0°C", could be calculated with confidence.

Plate 1. Upstream view of the experimental channel in the Wainui Valley (15 August 1971), with valley rim in backgound. (Photograph by T.K. Crosby).

## METHODS

### Description of the experimental channel

Reliable sampling of larval simuliids is usually difficult because of the heterogeneous nature of stream beds. Methods that have been used are summarized by Disney (1972). Shovel (Maitland & Penney 1967) and Surber (Kačanski 1970) samplers can be used for uniform small-stone stream beds, but such streams are infrequent. Removal of vegetation to which larvae were attached was used as one of three methods by Obeng (1967), and a box sampler was used to enclose vegetation on which larvae were attached by Ladle et al. (1972). If stones are removed as sampling units, larvae on them can be washed off into dishes (Obeng 1967; Chutter 1972) or collected by running fingers across the stone (Davies & Smith 1958). It was found that neither of the latter two methods removed all A. tillyardianum larvae from stones, and representative proportions of all larval instars were not always obtained as many small individuals were regularly missed. When artificial substrates are used, e.g. cones (Wolfe & Peterson 1959; Phelps & DeFoliart 1964), polythene tapes (Williams & Obeng 1962), wooden boards (Carlsson 1962), or mango leaves (Disney 1972), they are usually suspended in the water column, and it would appear that the larvae collected are those that have drifted downstream and colonized them. The larvae present may not necessarily be representative of populations occurring on natural attachment sites.

The small size of the Wainui Valley Stream precluded the use of most of the above sampling methods. Therefore, a 10 x 1 m experimental channel was constructed across a bend in the stream (Plate 1). The position of the channel was chosen to minimize interference from grazing stock which were in the paddock alongside the stream throughout most of the study.

a

b

Plate 2 (a). Entrance of experimental channel (15 August 1971), with stones controlling the amount of water entering from the stream. By October about a third of the stream flow was required to maintain similar levels in the channel, and the stones were used to divert water into the channel. The arrow indicates the oviposition stone used to assess egg laying activity of females.

(b). Riffle area below the experimental channel (15 August 1971), downstream sides of the stones are normally utilized as pupation sites. Grazing stock occasionally cross at this point. (Photographs by T.K. Crosby).

Once the level of the channel bed had stabilized, the remaining small basalt stones of the bed were supplemented with 40-80 mm diameter basalt stones that had been quarried about six months previously. Although the quarried stones had angled rather than rounded sides, this did not affect colonization. Complete uniformity of the bed of the channel was not attained; however, the three large stones left at the top (2) and bottom (1) tended to reduce scouring during periods of increased water flow.

Water flow through the channel was partly regulated to give a current of 0.4-0.6 m $s^{-1}$ by positioning large stones at the entrance to the channel (Plate 2a). Water level throughout the study was relatively constant and a "flood" increased the level from about 0.1 m average depth to about 0.3 m. The level of the stream varied about 0.6 m over the same period.

Colonization of the channel was allowed to occur naturally and, by the time sampling began seven months later, most species were present at densities comparable to those in riffle areas in unmodified parts of the stream. In fact, by the end of the sampling period the general appearance of the experimental channel was indistinguishable from unmodified areas of the stream apart from the lack of large stones. Thut (1969) found that the experimental channels he used to study Trichoptera also closely resembled natural areas.

Estimates of the number of larvae present in the experimental channel and in similar unmodified areas of the stream were made on three occasions. Although stones sampled from the unmodified areas were similar in size to those in the experimental channel, it was difficult to ensure complete comparability between areas because of the heterogeneity of the stream bed in natural situations. There was, however, good correspondence between these estimates on each sampling date (Fig. 3).

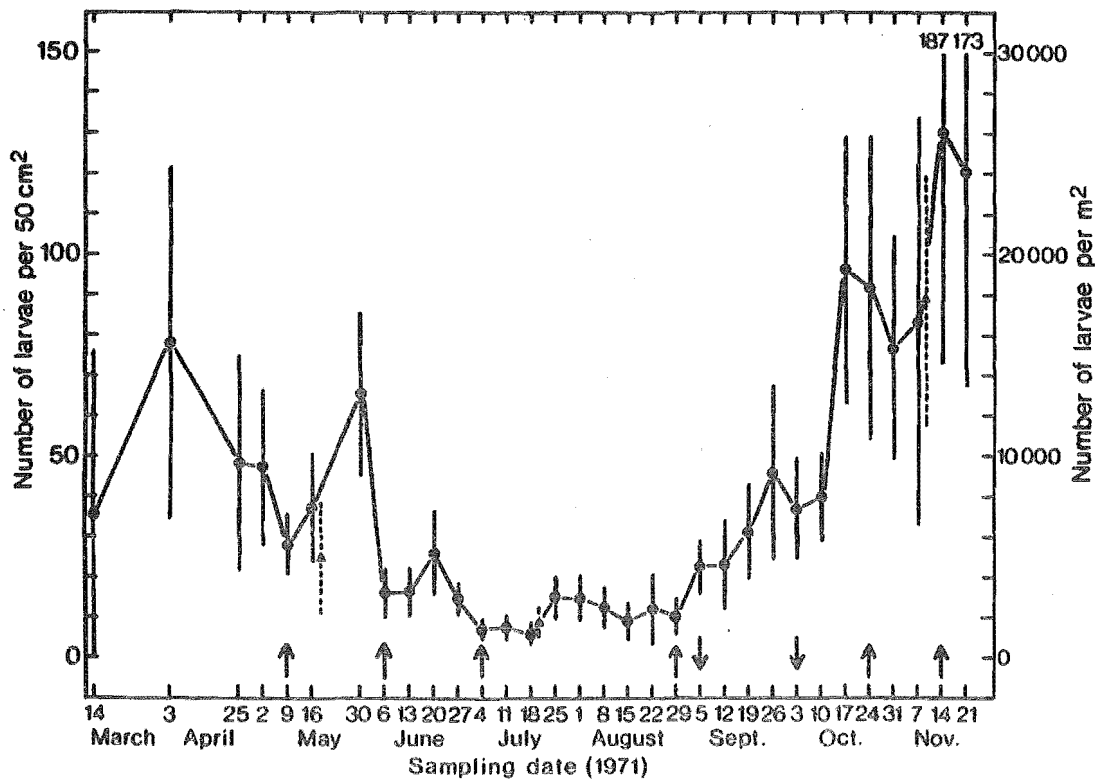


Fig. 3. Estimates of the mean number of larvae per 50 $cm^2$ (and larvae per $m^2$) on sampling dates with 95% confidence intervals of means. ●, experimental channel estimate; ▲, corresponding unmodified stream area estimate; ⇧, weeks in which water level increased; ⇩, human interference to experimental channel (partial damming).

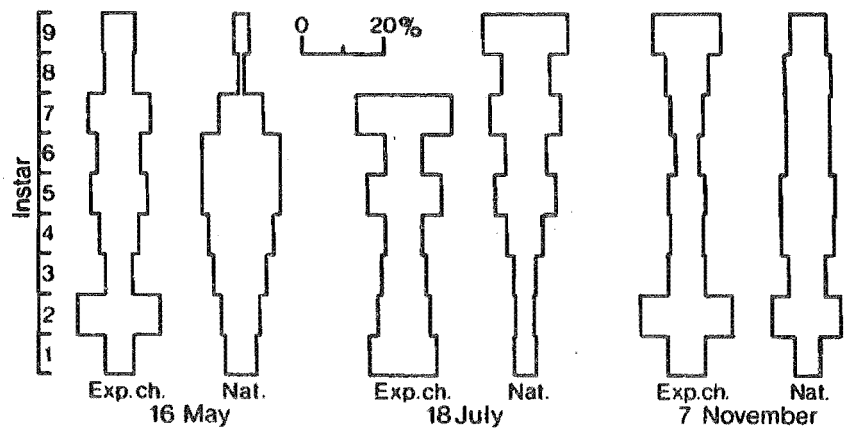


Fig. 4. Comparison of the percentage composition of larval instars of samples between the experimental channel (Exp. ch.) and unmodified areas of the stream (Nat.) on three sample dates.

The general percentage composition of larval instars in the samples was also similar, but there were some differences (Fig. 4). On 16 May the main difference was in the abundance of instar 2 larvae. In terms of numbers they were about three times as abundant in the experimental channel, whereas all other instars were present in approximately similar numbers in the two areas. The difference on 18 July was that instars 8 and 9 were in such low numbers in the experimental channel that they were not recorded. This probably was caused by high water levels shifting the small stones. In unmodified areas, however, the small stones taken as samples were probably protected from the full force of the current by the surrounding large stones. The first three instars were about 1.2-2.0 times more abundant in the experimental channel. Instar distributions were similar on 7 November, but with 9th instar larvae being about twice as numerous in the experimental channel.

It was concluded therefore that in general population changes indicated by the experimental channel were comparable with those occurring in unmodified areas of the stream. This was expected because when females return to the stream to lay it is unlikely that they would lay at the same place they hatched. Thus if large numbers had hatched from one area of the stream, females would lay throughout the length of the stream and some degree of uniformity between areas would therefore be expected.

Sampling

Sampling was carried out between March and November 1971, and was undertaken weekly from the end of April. The sampling unit employed was a stone (Crosby in prep.). One or two stones were usually sufficient to give a sample of 40-60 $cm^2$ potential attachment area.. These were collected at random from areas with suitable water flow for simuliids. From June-

November, 15-18 samples were taken on each sampling day. This number was calculated so that mortality within a cohort due to sampling would not exceed 5%. Most samples for immature stages were obtained from the experimental channel although on three occasions comparative samples were also taken from unmodified areas of the stream. Stones were placed in plastic pots with a small amount of water, and on return to the laboratory 2-4 h later, absolute ethanol was poured into the pots to preserve all animals. Samples were sorted and animals counted with the aid of a dissecting microscope, usually within a week of collection.

Instars of all A. tillyardianum larvae were determined, and pupae were sexed by examining the cephalic apotomes (Crosby in press "a"). Since pupal exuviae are normally retained within the pupal cocoon for 1-2 weeks after hatching the sex ratio of adults recently hatched could also be assessed from cephalic apotomes.

Egg laying activity of females was assessed by counting the number of egg masses that had been laid on a rock at the top of the channel (Plate 2a). Females lay only on certain rocks, and this was the main ovipositional rock in the channel area. Peaks and troughs in egg laying activity could be related to preceding peaks and troughs in numbers of pupae.

Stream temperatures were recorded with a mercury thermometer and water levels in the stream and channel were noted on each sampling day. Photographic records of the stream and channel were also made each time for later reference.

To determine the potential attachment area on a stone for larval simuliids (Maitland & Penney 1967), the top and two sides of each sample

stone were traced onto a sheet, and the area was measured with a planimeter.

At the conclusion of the sampling period, about a third of the samples were reexamined to check the accuracy with which A. tillyardianum larvae were assigned to instars and total numbers present. Very few larvae were reassigned to another instar, and most that were came from samples collected between June and August when numbers were low. Likewise, most counts of total numbers in samples were correct; the few incorrect totals were for samples collected between September and November when numbers were high.

## ANALYSIS OF DATA

Original numbers of larvae of all instars in a sample were transformed to numbers in terms of a standard 50 $cm^2$ potential attachment area sample. Mean numbers per 50 $cm^2$ and standard errors of the mean were calculated for every instar of a sampling date but only those of the cohorts extracted (see below) are presented in Tables 1-10. All original data are available in the author's thesis at the University of Canterbury, in the Department of Zoology, University of Canterbury, and in the British Museum (Natural History) Library.

### Separation of cohorts

For this study, a cohort is defined as larvae of a particular instar that could be identified in samples because of their low or high numbers compared with adjacent instars, and whose development could be traced from sample date to sample date. However, boundaries of cohorts were not clear-cut as all larval instars were collected on every sampling occasion.

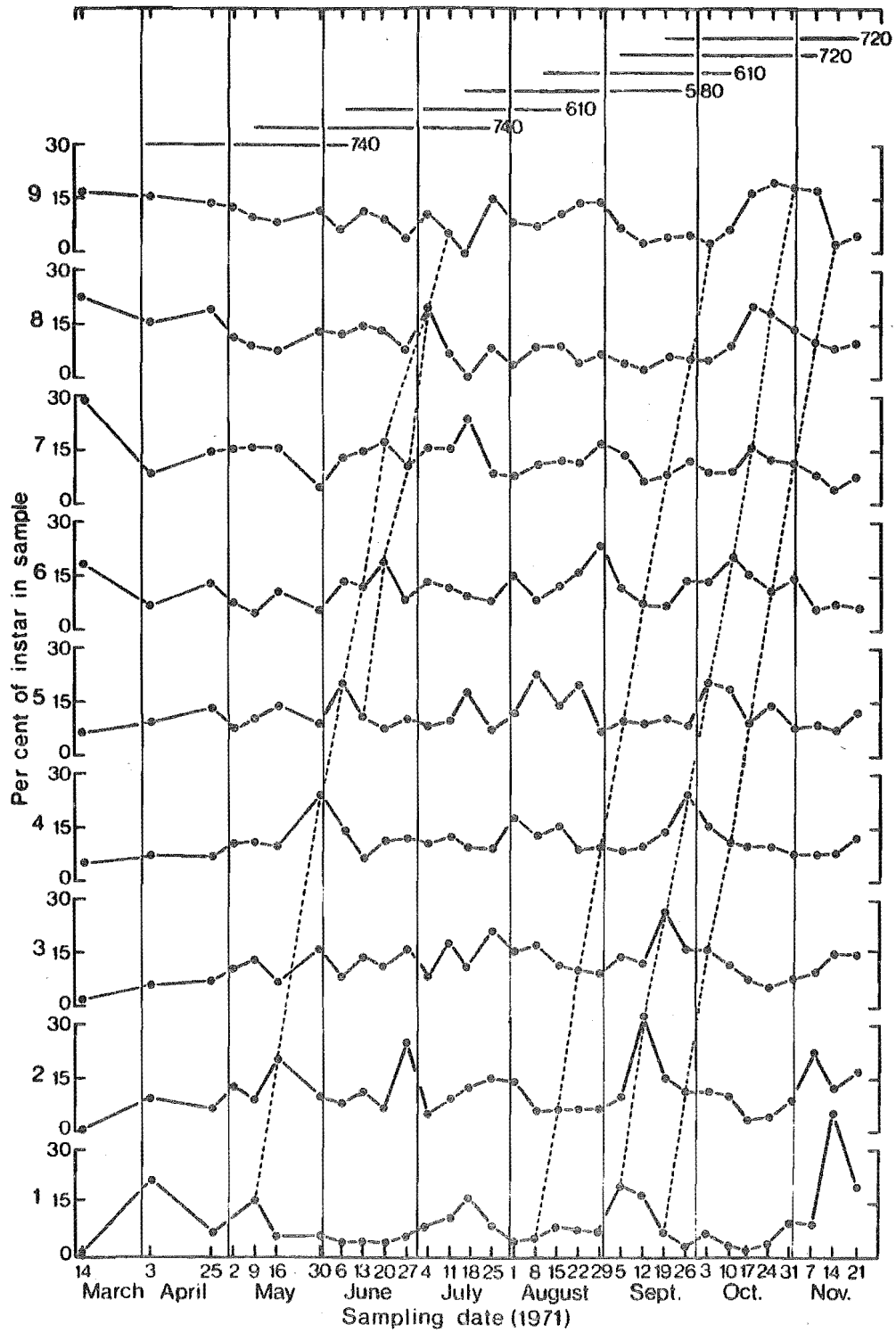


Fig. 5. Percent composition of the larval instars in samples on sampling dates. Dashed lines join larval instars of a cohort (note, only representative cohorts joined). Solid lines at the top indicate development times for some cohorts from instar 1 to pupa, and the number for each line is the time in "accumulated degree-days above 0°C" for development from egg to adult.

To aid in separating cohorts, the following procedure was adopted to adjust for mortality between instars. The numbers of larvae in each instar collected throughout the sampling period were summed to give total numbers for each instar. By adding these totals a combined total of larvae collected was obtained. The combined total was then divided by nine to give an "expected" number of larvae for each instar for the sampling period. The "expected" number of larvae per instar was divided by the actual number to give a weighting fraction for each instar. For every sampling date, the mean number of larvae in each instar was multiplied by the appropriate weighting factor to give "expected" mean numbers. Finally, using the "expected" mean numbers, the per cent composition was calculated for every sampling date and plotted (Fig. 5). In this way regular progressions of "peaks" and "troughs" were obtained (Fig. 5), and these could be traced from sample date to sample date. Cohorts could therefore be identified and changes in numbers from instar to instar could be calculated (Tables 1-10). "Peaks" and "troughs" remained reasonably discrete between sample dates, indicating instars were living for similar periods of time. Using this method, nine reasonably distinct cohorts could be traced through their life histories (Tables 1-9) and two incomplete cohorts could also be traced (Table 10). Four of these cohorts are indicated on Fig. 5.

## Effectiveness of placing larvae into size categories to follow population changes

Recently Jedlička (1972) compared various methods of determining age structure in collections of larvae, using Simulium (Odagmia) ornatum, and showed that different methods gave different population structures. In the present study, three methods were compared with a known instar distribution of A. tillyardianum, and each gave an entirely different

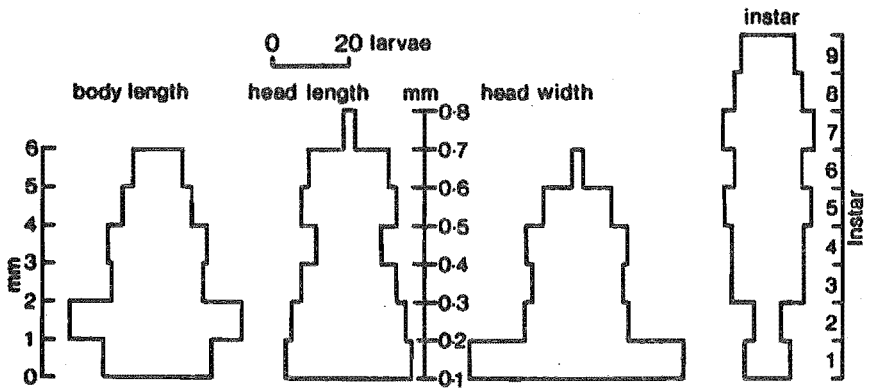


Fig. 6. Comparison of three methods of placing 154 *A. tillyardianum* larvae into size categories according to divisions of a measurement, and the instar categories for the larvae based on morphological and biometrical evidence.

result (Fig. 6). For example, if I had attempted to trace population changes by placing larvae into categories according to head capsule widths, as done by Davies & Smith (1958) for the univoltine species Prosimulium (Prosimulium) hirtipes and Rühm (1970b) for the multivoltine species Simulium (Boophthora) erythrocephalum (De Geer), I would not have detected any noticeable changes between collections. Early instars would have been lumped and the final instar split. In fact none of the methods of dividing measurements into groups gave reasonable approximations of the population structure (in terms of instars) at all! For this reason it is difficult, and probably misleading, to compare the results of most previous studies with my results.

## LIFE HISTORY OF A. TILLYARDIANUM

A female lays 250-330 eggs in a single layer mass measuring 6-8 mm by 3-5 mm on the downstream surface of stones about 15-100 mm below water level. The tops of oviposition stones protrude above water level, and they are located in riffle areas where currents are about 0.4-0.6 m $s^{-1}$. Usually oviposition stones are 0.1-0.2 m wide so that a reduced current rather than still water is present on the downstream side of the stones. All eggs in an egg mass hatch 6-8 days after being laid. Delayed hatching, such as that recorded for Cnephia pecuarum (Riley) by Bradley (1935), Simulium (Simulium) argyreatum Meigen by Grenier (1944, 1949), and for several species by Fredeen (1959), was not observed. It is probable that since adults are present at all times, any complete losses of young larvae over a two or three week period due to floods can be replaced by newly emerged adults.

First instar larvae drift downstream from oviposition sites until

they find suitable attachment areas. As they can survive and grow in slower currents than other instars, many of these larvae do not drift to areas where they can survive as later instars until they reach second, third or fourth instar (see Tables). This is similar to the situation found for Simulium (Boophthora) erythrocephalum by Rühm (1970a) where the main dispersal stage was instar 2, Kačanski (1970) for Simulium (Odagmia) ornatum and by Chutter (1972) for Simulium (Eusimulium) nigritarse Coquillett.

Larvae are usually found on the top and upstream sides of stones, as described for Scottish Simuliidae by Maitland & Penney (1967). The larvae are regularly spaced on the stones because each larva maintains an "individual space". Any intrusion into this space by an object will invoke an attack reaction if it touches a larva (Tonnoir 1925; Disney 1972). A. tillyardianum larvae display this territorial behaviour from the time of hatching and it is for this reason that mats of larvae completely covering stones are not found. High densities, in the order of 100 larvae $cm^{-2}$ found in the Australian species Austrosimulium (Novaustrosimulium) bancrofti (Taylor) (M. Colbo, pers. comm.), and some North American species (Cameron 1922; Sommerman et al. 1955; Abdelnur 1968), therefore do not occur in A. tillyardianum.

Although larvae of all sizes utilize the same food source (microseston of Maciolek & Tunzi (1968)) they do not appear to be competing. This may be because different instars filter slightly different levels of a water column according to their size. Each instar lasts for a similar period of time as shown by laboratory rearing. In the sampling period this was about one week as indicated by "peaks" and "troughs" in Fig. 5.

Simuliid larvae do not appear to move from attachment sites unless

conditions become intolerable (W.H.O. Expert Committee on Onchocerciasis 1966; Minshall & Winger 1968; Kureck 1969; Tarshis & Neil 1970). After the disturbance to the experimental channel on 3 October, when it was partially dammed, many of the larvae collected had half-emptied guts indicating that the current in the channel was not sufficient to allow feeding to occur. Thus, this disturbance which changed part of the experimental channel to an unsuitable habitat for A. tillyardianum larvae was not sufficient to cause most of them to drift out of the area. The tolerance of larvae to disturbance is such that stones can be removed from the water for up to 30 s and then replaced with only 10-20% of the larvae detaching. This attachment tenacity also indicates that collecting samples with nets or a Surber-type sampler would not be very successful in collecting all larvae of A. tillyardianum, and is one reason why stones were adopted as sampling units in this study.

Pupation normally occurs on downstream sides of stones. In the experimental channel, the small stones were not entirely suitable for pupation, and a considerable proportion of instars 8 and 9 drifted out of the channel to pupate on large stones in unmodified stream areas (Plate 2b). Hatching of adults occurs about a week after pupation, and they immediately disperse. No adults were sweepnetted in the vicinity of the stream during the sampling period, and no males were collected even though the sex ratio at hatching is about 1:1. Females caught landing on me were nearly all parous, as shown by the presence of follicular relics (Davies 1961). Nulliparous females are probably autogenous, but no detectable ovarian development was found in laboratory-hatched specimens over 3-4 days; copulation may first be required. It is not known what proportion of females undergo more than one ovarian cycle, but in two Canadian species of Prosimulium the rate was about 10-20% (Davies 1961).

## RESULTS AND DISCUSSION

### Developmental times of cohorts

Maximum stream temperatures usually occur between 1300-1500 h (Hopkins 1971), hence the stream temperatures I recorded would usually be daily maxima. According to Hopkins (1971: Table II) average stream temperatures in a small New Zealand stream were usually 1-2°C lower than the maximum stream temperature recorded, depending on season. Therefore, if the temperature of the Wainui Stream was above 10°C during the autumn and spring months, 2°C was subtracted to give an average stream temperature, and if the stream temperature was below 10°C as during the winter months, 1°C was subtracted. These temperatures were then multiplied by the number of days between samples (7) and summed for all stages to give developmental times in terms of "accumulated degree-days above 0°C" (Davies & Smith 1958).

Estimated developmental times for A. tillyardianum cohorts varied between 580-740 degree-days (Fig. 5). The lower developmental times of cohorts were associated with relatively uniform temperatures throughout the period of growth, whereas the higher developmental times were associated with cohorts growing through periods of falling or rising temperatures (Figs. 2, 5).

The estimates for A. tillyardianum are similar to those of 650 and 700 degree-days found for Prosimulium (Prosimulium) hirtipes by Davies & Smith (1958), and of 800 degree-days for Simulium (Simulium) venustum Say by D.M. Davies (1950). However, they are larger than the 190-230 degree-days estimated for Simulium (Psilozia) vittatum Zetterstedt growing at 17-27°C (Becker 1973). The estimated development time for a mixed population of Prosimulium (Prosimulium) fuscum Syme & Davies and P. (P.)

Table 1. Peak cohort, early estimates probably inaccurate.

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Change in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 3 April | 1 | 25.0 | -12.3 | 62.3 | 64.6 | | 1.00 | Probably inaccurate estimate |
| 25 April | 5 | 11.2 6.7 | 1.02 | 12.47 | 38.1 | -13.8 | 0.45 | |
| | 6 | 4.5 | -0.71 | 9.68 | 52.0 | | | |
| 2 May | 6 | 5.5 2.2 | 0.57 | 3.77 | 33.1 | -5.7 | 0.22 | natural decline? |
| | 7 | 3.3 | 1.27 | 5.25 | 27.4 | | | |
| 9 May | 7 | 2.0 | 0.39 | 3.70 | 36.8 | -3.5 | 0.08 | |
| 16 May | 7 | 4.3 2.6 | 0.47 | 4.77 | 35.6 | +2.3 | 0.17 | |
| | 8 | 1.7 | 0.05 | 3.25 | 42.1 | | | |
| 30 May | 9 | 4.0 | 0.92 | 6.99 | 34.9 | -0.3 | 0.16 | high water levels |
| 6 June | Pupa | 0.37 | 0.01 | 0.73 | 45.8 | -3.63 | 0.02 | larvae drifting out to pupate |

Table 2. Peak cohort, numbers reduced through high water level

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 9 May | 1 | 6.1 | 2.64 | 9.46 | 25.6 | | 0.32 | |
| 16 May | 2 | 11.8 | 8.17 | 15.45 | 13.4 | +5.7 | 0.63 | migration in from hatching sites |
| 30 May | 4 | 18.8 | 10.41 | 27.41 | 20.3 | +7.0 | 1.00 | |
| 6 June | 5 | 3.2 | 1.50 | 5.03 | 25.1 | -15.6 | 0.17 | effect of high water level |
| 13 June | 5 | 1.7 | 0.73 | 2.62 | 26.4 | -0.2 | 0.16 | |
| | 6 | 1.3 | 0.60 | 2.01 | 25.3 | | | |
| | 3.0 | | | | | | | |
| 20 June | 6 | 3.3 | 0.84 | 5.68 | 34.6 | +2.6 | 0.30 | migration in from upstream areas due to falling water level |
| | 7 | 2.3 | 0.69 | 3.85 | 32.4 | | | |
| | 5.6 | | | | | | | |
| 27 June | 7 | 0.6 | 0.04 | 1.12 | 43.2 | -5.0 | 0.03 | larvae drifting out to pupate |
| 4 July | 8 | 1.0 | 0.41 | 1.54 | 27.1 | +0.4 | 0.05 | |
| 11 July | 9 | 0.2 | 0.01 | 0.45 | 45.1 | -0.8 | 0.01 | |
| 18 July | Pupa | 0.1 | | | | | 0.005 | |

Table 3. Trough cohort.

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 6 June | 1 | 1.0 | 0.09 | 1.87 | 42.5 | | 0.20 | |
| 13 June | 2 | 3.2 | 1.44 | 4.92 | 25.7 | +2.2 | 0.63 | migration in from hatching sites |
| 20 June | 3 | 5.1 | 2.83 | 7.27 | 20.5 | +1.9 | 1.00 | |
| 27 June | 4 | 1.9 | 0.55 | 3.22 | 33.1 | -3.2 | 0.37 | |
| 4 July | 5 | 0.6 | 0.13 | 0.98 | 35.6 | -1.3 | 0.12 | effect of high water level |
| 11 July | 5 | 0.6 | 0.18 | 0.93 | 31.8 | +0.5 | 0.22 | |
| | 1.1 | | | | | | | natural decline through predation? territorial behaviour? |
| | 6 | 0.5 | -0.03 | 0.96 | 50.0 | | | |
| 18 July | 6 | 0.3 | 0.01 | 0.64 | 45.1 | -0.2 | 0.18 | |
| | 0.9 | | | | | | | |
| | 7 | 0.6 | -0.02 | 0.92 | 43.0 | | | |
| 25 July | 7 | 0.5 | 0.11 | 1.73 | 44.7 | -0.4 | 0.10 | |
| 1 August | 8 | 0.3 | 0.00 | 0.51 | 46.5 | -0.2 | 0.06 | |
| 8 August | 9 | 0.5 | 0.16 | 0.90 | 33.6 | +0.2 | 0.10 | |
| 15 August | Pupa | 0.10 | | | | -0.4 | 0.02 | drifting out to pupate |

Table 4. Peak cohort, numbers reduced through high water levels.

| Sampling date | Stage | | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|---|
| 27 June | 2 | 8.0 | 5.0 | 1.76 | 8.24 | 20.5 | | 1.00 | |
| | 3 | | 3.0 | 1.93 | 4.03 | 16.4 | | | |
| 4 July | 3 | 2.1 | 1.0 | 0.42 | 1.59 | 27.0 | -6.0 | 0.26 | effect of high water level |
| | 4 | | 1.1 | 0.33 | 1.83 | 32.4 | | | |
| 11 July | 3 | 3.1 | 2.0 | 1.10 | 2.90 | 21.1 | +1.0 | 0.38 | |
| | 4 | | 1.1 | 0.65 | 1.55 | 19.1 | | | |
| 18 July | 5 | | 0.9 | 0.26 | 1.58 | 33.6 | -2.2 | 0.11 | |
| 25 July | 5 | 1.5 | 0.8 | 0.26 | 1.43 | 32.8 | +0.6 | 0.19 | |
| | 6 | | 0.7 | 0.17 | 1.20 | 35.4 | | | |
| 1 August | 6 | | 1.3 | 0.15 | 2.46 | 42.1 | -0.2 | 0.16 | natural decline through |
| 8 August | 7 | | 0.6 | 0.13 | 1.04 | 37.0 | -0.7 | 0.07 | predation? |
| 15 August | 8 | | 0.5 | 0.04 | 1.01 | 43.5 | -0.1 | 0.06 | territorial behaviour? |
| 22 August | 9 | | 1.0 | -0.47 | 2.37 | 70.6 | +0.5 | 0.12 | |
| 29 August | Pupa | | 0.33 | 0.03 | 0.62 | 43.3 | -0.67 | 0.04 | larvae drifting out to pupate |

Table 5. Trough cohort

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 18 July | 1 | 1.8 | 0.46 | 2.15 | 30.2 | | 0.56 | migration in from hatching sites |
| 25 July | 2 | 3.2 | 2.11 | 4.19 | 15.7 | +1.4 | 1.00 | |
| 1 August | 3 | 3.2 | 2.31 | 4.10 | 13.3 | 0.0 | 1.00 | |
| 8 August | 4 | 1.9 | 0.71 | 3.11 | 29.7 | -1.3 | 0.59 | natural decline through predation? Territorial behaviour? |
| 15 August | 5 | 1.2 | 0.30 | 2.16 | 35.7 | -0.7 | 0.38 | |
| 22 August | 6 | 1.3 | -0.07 | 2.65 | 49.8 | +0.1 | 0.41 | |
| 29 August | 7 | 0.9 | 0.11 | 1.55 | 56.9 | -0.4 | 0.28 | effect of high water level |
| 5 September | 8 | 0.6 | 0.22 | 1.00 | 30.2 | -0.3 | 0.19 | human interference, partial damming of channel |
| 12 September | 9 | 0.3 | -0.05 | 0.73 | 53.9 | -0.3 | 0.09 | |
| 19 September | Pupa | 0.1 | | | | -0.2 | 0.03 | larvae drifting out to pupate |

Table 6. Trough cohort, affected by human interference, no effect on numbers

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 8 August | 1 | 0.8 | 0.10 | 1.43 | 41.2 | | 0.40 | |
| 15 August | 2 | 0.9 | -0.12 | 1.83 | 53.8 | +0.1 | 0.45 | migration in from hatching sites |
| 22 August | 3 | 2.0 | 0.64 | 3.27 | 31.8 | +1.1 | 1.00 | |
| 29 August | 4 | 1.4 | 0.67 | 2.14 | 24.8 | -0.6 | 0.70 | effect of high water level |
| 5 September | 5 | 1.8 | 1.19 | 2.33 | 15.3 | +0.4 | 0.90 | human interference, partial damming of channel |
| 12 September | 6 | 0.8 | 0.35 | 1.15 | 25.2 | -1.0 | 0.40 | (no effect!) |
| 19 September | 7 | 1.0 | 0.44 | 1.45 | 25.4 | +0.2 | 0.50 | |
| 26 September | 8 | 1.5 | 0.47 | 2.51 | 32.5 | +0.5 | 0.75 | |
| 3 October | 9 | 0.5 | 0.18 | 0.73 | 28.2 | -1.0 | 0.25 | human interference as above |
| 10 October | Pupa | 0.44 | 0.09 | 0.78 | 37.3 | -0.06 | 0.22 | larvae drifting out to pupate |

Table 7. Trough cohort, human interference

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 22 August | 1 | 1.4 | 0.65 | 2.20 | 25.8 | | 0.32 | |
| 29 August | 2 | 1.2 | 0.48 | 1.84 | 27.9 | -0.2 | 0.27 | migration in from hatching sites |
| 5 September | 3 | 4.4 | 3.00 | 5.81 | 15.1 | +3.2 | 1.00 | human interference, partial damming of channel |
| 12 September | 4 | 2.3 | 1.27 | 3.31 | 21.1 | -2.1 | 0.52 | |
| 19 September | 5 | 2.5 | 1.05 | 2.36 | 32.4 | +0.2 | 0.57 | |
| 26 September | 6 | 3.8 | 1.65 | 6.05 | 27.1 | +1.3 | 0.86 | migration in through lower water levels upstream |
| 3 October | 7 | 1.4 | 0.67 | 4.23 | 33.8 | -2.4 | 0.32 | human interference as above, sharp decline in numbers |
| 10 October | 8 | 2.4 | 1.03 | 3.82 | 27.2 | +1.0 | 0.55 | |
| 17 October | 9 | 11.5 | 6.03 | 16.88 | 22.5 | +9.1 | 2.61 | migration in from upstream through low water levels |
| 24 October | Pupa | 1.55 | 0.60 | 2.51 | 29.1 | -9.95 | 0.35 | larvae drifting out to pupate |

Table 8. Peak cohort, beginning from a sampling period in which human interference occurred.

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 5 September | 1 | 5.8 | 3.18 | 8.48 | 21.5 | | 0.43 | human interference no effect? |
| 12 September | 2 | 9.6 | 3.61 | 15.65 | 29.6 | +3.8 | 0.71 | |
| 19 September | 3 | 10.8 | 6.02 | 15.67 | 21.1 | +1.2 | 0.80 | migration in from hatching sites |
| 26 September | 4 | 13.5 | 6.89 | 20.14 | 23.2 | +2.7 | 1.00 | |
| 3 October | 5 | 7.0 | 3.78 | 8.42 | 18.0 | -6.5 | 0.52 | human interference, partial damming, numbers reduced |
| 10 October | 6 | 5.3 | 3.20 | 7.39 | 18.8 | -1.7 | 0.39 | |
| 17 October | 7 | 9.0 | 4.40 | 13.53 | 24.1 | +3.7 | 0.67 | migration in from upstream through low water levels |
| 24 October | 8 | 12.7 | 6.69 | 18.61 | 22.3 | +3.7 | 0.94 | |
| 31 October | 9 | 8.8 | 4.19 | 13.39 | 24.8 | -3.9 | 0.65 | larvae drifting out to pupate |
| 7 November | Pupa | 1.42 | 0.39 | 2.45 | 34.5 | -7.38 | 0.11 | trichopteran predation |

Table 9. Trough cohort after a major peak.

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 19 September | 1 | 2.6 | 1.67 | 3.53 | 16.9 | | 0.26 | migration in from hatching sites |
| 26 September | 2 | 8.2 | 4.39 | 12.05 | 22.1 | +5.6 | 0.82 | |
| 3 October | 3 | 8.0 | 5.10 | 10.87 | 17.1 | -0.2 | 0.80 | human interference, partial damming of channel |
| 10 October | 4 | 5.9 | 3.95 | 7.90 | 15.8 | -2.1 | 0.59 | |
| 17 October | 5 | 10.0 | 6.42 | 13.67 | 17.1 | +4.1 | 1.00 | migration in from upstream through low water levels |
| 24 October | 6 | 7.5 | 2.98 | 12.07 | 28.6 | -2.5 | 0.75 | |
| 31 October | 7 | 4.7 | 2.04 | 7.45 | 27.0 | -2.8 | 0.47 | |
| 7 November | 8 | 5.3 | 2.79 | 7.88 | 22.6 | +0.6 | 0.53 | higher water level? |
| 14 November | 9 | 1.3 | 0.23 | 2.38 | 39.1 | -4.0 | 0.13 | larvae drifting out to pupate |
| 21 November | Pupa | 0.58 | 0.17 | 0.99 | 33.6 | -0.72 | 0.06 | |

Table 10a. Incomplete peak cohort

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 3 October | 1 | 3.2 | 1.87 | 4.61 | 20.1 | | 0.23 | human interference, partial damming of channel |
| 10 October | 2 | 7.1 | 5.19 | 9.05 | 12.8 | +3.9 | 0.50 | migration in from hatching sites |
| 17 October | 3 | 13.9 | 8.11 | 19.74 | 19.8 | +6.8 | 0.98 | migration in from upstream through low water levels |
| 24 October | 4 | 14.2 | 6.71 | 21.64 | 25.0 | +0.3 | 1.00 | |
| 31 October | 5 | 5.9 | 2.80 | 9.04 | 25.0 | -8.3 | 0.42 | |
| 7 November | 6 | 3.0 | 0.49 | 5.60 | 39.8 | -2.9 | 0.21 | natural decline, predation? |
| 14 November | 7 | 2.0 | 0.86 | 3.04 | 26.4 | -1.0 | 0.14 | territorial behaviour? |
| 21 November | 8 | 3.3 | 1.70 | 4.89 | 23.0 | +1.3 | 0.23 | |

Table 10b. Incomplete trough cohort

| Sampling date | Stage | Mean number | 95% confidence limits of mean | | Per cent standard error of mean | Changes in numbers between sample dates | Mean number as a proportion of highest early instar | Causes of change |
|---|---|---|---|---|---|---|---|---|
| 10 October | 1 | 2.0 | 1.12 | 2.97 | 21.5 | | 0.20 | migration in from hatching sites and upstream areas through low water levels |
| 17 October | 2 | 6.9 | 4.03 | 9.77 | 19.7 | +4.9 | 0.70 | |
| 24 October | 3 | 9.8 | 5.53 | 14.15 | 20.8 | +2.9 | 1.00 | |
| 31 October | 4 | 8.2 | 4.20 | 12.13 | 23.0 | -1.6 | 0.84 | |
| 7 November | 5 | 6.3 | 1.64 | 10.81 | 34.9 | -1.9 | 0.64 | natural decline |
| 14 November | 6 | 4.8 | 2.19 | 7.33 | 25.6 | -1.5 | 0.49 | predation? territorial behaviour? |
| 21 November | 7 | 3.8 | 0.89 | 6.78 | 36.4 | -1.0 | 0.39 | |

mixtum Syme & Davies was 1100 degree-days according to Davies & Syme (1958), a figure which is considerably larger than any other estimate.

Because of the vegetation change in the Wainui Valley from forest to pasture land, it is possible that higher water temperatures, especially in summer, now prevail than formerly (Gray & Edington 1969). As a result, available development times for stream invertebrates may have increased in terms of degree-days above 0°C and allowed more individuals to be produced in the same period of time.

Factors affecting larval numbers

Two main factors appeared to influence population changes of A. tillyardianum larvae in the experimental channel of the Wainui Valley Stream: water level and larval behaviour (Tables 1-10).

In some simuliid populations, predators and parasites have been reported to be important population controlling factors. This is not so for A. tillyardianum. Few larvae were eaten by predators (Crosby in prep.), and no parasites were found. In some studies nematodes (Enoplida: Mermithidae) were found to be common parasites of larvae, 30-100% of the larvae collected being infected (Doby & Laurent 1953; Hocking & Pickering 1954; Fredeen & Shemanchuk 1960; Anderson & Dicke 1960; Anderson & DeFoliart 1962; Phelps & DeFoliart 1964, Welch 1964; Rubzov 1967) and microsporidia have also been reported to be common parasites of simuliids (Jenkins 1964; Weiser 1964; Jamnback 1970). A commensal trichomycete fungus, Harpella melusinae Léger & Duboscq, was common in the digestive tracts of A. tillyardianum larvae (Crosby in press "b") but had no apparent effects on them. Cannibalism of small larvae by large larvae did not appear to occur in A. tillyardianum, although this has been reported by

Burton (1971) for Simulium (Edwardsellum) damnosum Theobald and was stated to be an important mortality factor at times in Simulium (Eusimulium) nigritarse by Chutter (1972).

1. Water level

High water levels in the channel reduced larval numbers (Fig. 3, Tables 1-10). Probably the small stones of the bed were moved around causing larvae to detach and drift downstream. Some larvae may have reattached to the large stones of the unmodified areas and therefore survived the floods, but many would have perished. The main increases in water level occurred in the sampling periods preceding 6 June and 4 July, and on both occasions numbers were reduced about fourfold. The importance of floods in reducing simuliid numbers has been shown by several authors (Allen 1951; Wolfe & Peterson 1959; Cariaso 1962; Maitland & Penney 1967; Chutter 1972) but the extent of the reductions were not quantified. Some pupae may have died through crushing when stones were moved around on the stream bed although this was not observed.

Larvae of all sizes appeared to be equally affected by high water levels. This was shown by the fact that the percentage composition on sampling dates after floods was similar to the expected composition if floods had not occurred (Fig. 5).

In contrast, lower water levels in the stream increased numbers in the channel. As the top parts of large stones in the stream were gradually exposed, larvae living on these sites were forced to leave, and it appears as if many larvae from above the channel migrated into the channel for this reason. Such migrations occurred particularly in the sampling periods preceding 20 June, 26 September and 17 October (Fig. 3), immigrants on the latter date being predominantly large larvae. In unmodified areas of

the stream, lower water levels and slower currents allowed growths of algae and diatoms and deposits of sediment to occur on many stones. These must have reduced the number of potential attachment sites for larvae in these areas (Zahar 1951; Fee 1967).

Fluctuations in water level occurring in the stream were not sufficient to cause mortality due to desiccation of egg masses (Smart 1934; Poole 1967; Rühm 1969) or to stranding of pupae (Cameron 1922; Mackerras & Mackerras 1948; Fredeen & Shemanchuk 1960). In fact, if there was any mortality due to desiccation, it would have been caused solely by human disturbance.

2. Behaviour of larvae

Superimposed upon the changes caused by fluctuations in water level were those caused by behaviour of larvae. Larvae maintain territories from the time they hatch, and the size of a larva determines the size of a territory. This territorial behaviour has an important effect on larvae migrating into an area and seeking attachment sites. If mainly large larvae are present, representing a "peak" cohort (Fig. 5), few small larvae will be able to settle. However, as soon as the large larvae pupate, their territories can be taken over by many small larvae which normally would be the offspring of a "trough" cohort (Fig. 5). Thus, by the time offspring of the "peak" cohort are seeking attachment sites, most sites would be occupied by offspring of the "trough" cohort, and therefore a considerable proportion of the "peak" cohort offspring might not survive. The "trough" cohort offspring, therefore, would become the next "peak" cohort. This factor is particularly important at higher population densities and when there are few favourable attachment sites at the times of reduced water flow.

Interspecific competition for space appeared to be unimportant during the period of study. In areas of faster water flow Neocurupira chiltoni (Campbell) larvae (Diptera: Blephariceridae) occasionally appeared to occupy sites suitable for A. tillyardianum, but N. chiltoni numbers were generally low. Cased Trichoptera larvae were common but did not occupy the same area of stones as A. tillyardianum larvae. Rather, they reduced the number of available pupation sites for A. tillyardianum and may have caused some mature larvae to leave the channel.

As well as larvae migrating into and out of the channel in response to changes in water level, young larvae migrated into the channel from their hatching sites, and maturing larvae left to find suitable pupation sites.

In most of the cohorts traced (Tables 1-10) there was a gradual build-up in numbers until about the fourth instar. Similar increases have been reported by several other authors (Balay 1964; Rühm 1970a, Kaćanski 1970; Chutter 1972). Presumably the small larvae entering the channel were coming from marginal areas where they were able to survive. Under laboratory conditions first instar larvae could survive at least a week in still water. Most differences from this pattern of build-up in numbers could be attributed to the effects of high water level. The necessity of early instars to find suitable attachment sites appears to make this stage of the life history the most crucial and hazardous of all, since most favourable attachment sites are usually occupied by larger larvae. Failure to find an attachment site might eventually result in larvae drifting out of the stream altogether.

A large number of mature larvae left the channel to pupate. Thus, in spite of a large influx of large larvae into the channel on 17 October,

few pupated in the area (Tables 7, 8). Pupation usually occurs on the downstream sides of stones where currents are reduced (Maitland & Penney 1967) and there is less risk of larvae being swept away during the process of pupation. The small stones of the channel did not entirely fulfil these conditions, thus it was not surprising that most larvae pupated elsewhere.

Factors affecting pupae and adults

Most pupae in the experimental channel hatched. A few were eaten by Trichoptera (Crosby in prep.) but none were found to be parasitized.

Although adult mortality was not observed, it undoubtedly occurred and several possible means can be suggested. No adults were found drowned through entanglement in their pupal exuviae, such as reported for Simulium (Edwardsellum) damnosum by Burton (1966). On hatching, adults rise to the water surface enclosed in a form-fitting layer of air, then fly away. Under laboratory conditions it was noted that adults paused for several seconds on the water surface before flying away. During this pause some may be drenched by water splashes and drowned. Predation by spiders and fantails (Rhipidura filiginosa Sparrman (Passeriformes: Muscicapidae)) is also likely. Some may die through heavy rain wetting them, but low temperatures do not appear to kill them since females were seen laying on the oviposition stone in the channel during the coldest part of the year. Some females may die when laying their eggs by being trapped by adjacent egg masses (Smart 1934; Poole 1967) or by wetting when they rise to the water surface again.

Survival rate

The survival rate of A. tillyardianum appeared to be high (Tables 1-10). Even if only half the females of a cohort hatching in the experimental channel survived to lay one egg mass, enough larvae would hatch to more than replace that cohort. Since it appeared as if only a half to a tenth of a cohort actually pupated in the channel, the other members of a cohort pupating in other areas would have contributed a further large number of larvae to the replacement cohort. This reproductive potential explains the ability of the species to increase in numbers in spite of sometimes heavy losses due to high water levels, and also its ability to colonize new areas quickly when these become available.

## SUMMARY

Population changes of a common New Zealand simuliid, Austrosimulium (Austrosimulium) tillyardianum Dumbleton, were investigated using a 10 x 1 m experimental channel in the Wainui Valley Stream, Banks Peninsula. A. tillyardianum was the only simuliid species in the stream, and all nine larval instars were present throughout the seven month study period. In this time, nine cohorts were traced through their life histories, and the results obtained appeared comparable to the population changes occurring in unmodified areas of the stream.

Population changes in the experimental channel were affected by the following factors:

(1) Water level; high water levels reduced the number of larvae, probably through shifting stones of the stream bed. Larvae of all sizes appeared to be equally affected by high water levels. Lower water levels increased

the number of larvae in the channel because larvae from upstream areas migrated in. Fluctuations in water level were not sufficient to allow egg masses or pupae to be desiccated.

(2) Behaviour of larvae; larvae were regularly spaced on stones and maintained territories. Small larvae drifted into the channel from upstream oviposition sites, and usually peak numbers of a cohort were not found until the third or fourth instar. If many large larvae were present, few small larvae could settle in these areas. A considerable proportion of mature larvae left the channel to pupate downstream.

(3) Predators were unimportant, and no parasites were found. Interspecific competition for attachment sites rarely occurred.

The survival rate of A. tillyardianum appeared to be high, and allows it to increase in numbers and colonize new areas quickly.

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Paper 4

# Food of the New Zealand trichopterans *Hydrobiosis parumbripennis* McFarlane and *Hydropsyche colonica* McLachlan

## Summary

The food of the larvae of two New Zealand Trichoptera, *Hydrobiosis parumbripennis* and *Hydropsyche colonica*, was investigated between March and November 1971 at the Wainui Valley Stream, Canterbury, New Zealand. Most samples were collected from an experimental channel constructed in the stream in 1970.

The first three instars of *H. parumbripennis* were mainly detrital feeders, whereas the two later instars were exclusively carnivorous. In contrast, *H. colonica* was omnivorous.

The main prey taken by *H. parumbripennis* were larvae of a simuliid, *Austrosimulium tillyardianum*, Chironomidae (subfamily Orthocladiinae), and an ephemeropteran, *Deleatidium* sp. The first three instars appeared to select chironomids in preference to simuliids, whereas the converse was true for the final two instars. The main prey taken by *H. colonica* was *A. tillyardianum*.

The size of prey and the mean number of prey found per larva of *H. parumbripennis* increased with each instar. Forage ratios indicated that *H. parumbripennis* were eating *A. tillyardianum* at the same relative frequency as their abundance in the fauna, but that the chironomids and *Deleatidium* sp. were being preferentially selected as prey. Cased caddis larvae were avoided as food items, as was the mollusc *Potamopyrgus antipodarum*. Although *H. parumbripennis* was the most important predator of *A. tillyardianum* in the stream, it is considered that it has little effect on the simuliid population.

## Introduction

The feeding habits of larvae of New Zealand Trichoptera are poorly known. In this paper, the food of two species is reported; that of a primarily carnivorous species *Hydrobiosis parumbripennis* McFarlane, 1951 (Rhyacophilidae), and an omnivorous species *Hydropsyche colonica* McLachlan, 1871 (Hydropsychidae). The study was carried out at the Wainui Valley Stream, Banks Peninsula, Canterbury between March and November 1971, and forms part of an investigation on the population changes of a common simuliid, *Austrosimulium (Austrosimulium) tillyardianum* Dumbleton, 1973 (Crosby, in prep.). Since Trichoptera larvae appeared to be the only

TABLE 1. Percentage composition of the fauna collected in the Wainui Valley Stream between March and November 1971. An asterisk (*) before the species name indicates a carnivorous species. A plus (+) indicates that the species made up < 0.05% of the total fauna collected

| | | % of fauna collected |
|---|---|---|
| INSECTA | | |
| DIPTERA | | |
| Simuliidae | *Austrosimulium tillyardianum* Dumbleton | 45.05 |
| Chironomidae | larvae of 2 Orthocladiinae spp. | 29.30 |
| Blephariceridae | *Neocurupira chiltoni* (Campbell) | 2.59 |
| Tipulidae | *Tasiocera* sp. | 0.28 |
| TRICHOPTERA | | |
| Helicopsychidae | *Helicopsyche* sp. | 5.35 |
| Sericostomatidae | *Pycnocentrodes aureola* (McLachlan) | 12.59 |
| | *Pycnocentria evecta* McLachlan | 1.92 |
| | *Olinga feredayi* (McLachlan) | + |
| Leptoceridae | *Hudsonema amabilis* (McLachlan) | + |
| Hydropsychidae | **Hydropsyche colonica* McLachlan | 0.05 |
| Rhyacophilidae | **Hydrobiosis parumbripennis* McFarlane | 0.27 |
| PLECOPTERA | | |
| Gripopterygidae | *Zelandoperla maculata* (Hare) | 0.63 |
| EPHEMEROPTERA | | |
| Leptophlebiidae | *Deleatidium* sp. | 1.07 |
| Siphlonuridae | *Coloburiscus humeralis* (Walker) | + |
| ARACHNIDA | | |
| ACARINA | Species of Hydrachnida | 0.08 |
| MOLLUSCA | | |
| Hydrobiidae | *Potamopyrgus antipodarum* (Gray) | 0.77 |
| COELENTERATA | **Hydra* sp. | + |
| PLATYHELMINTHES | **Dugesia montana* Nurse | + |
| ANNELIDA | | |
| OLIGOCHAETA | immature | + |

potential invertebrate predators present in the stream during the investigation period, the main purpose of this aspect of the study was to discover if the simuliid population was influenced by the trichopterans.

As few species were present in the riffle areas of the Wainui Valley Stream (Table 1), it was possible to identify accurately most of the animals eaten by trichopteran larvae. In the case of the main prey species, *A. tillyardianum*, larval instars could be identified and this allowed some information on the relationship between size of predator and size of prey to be obtained.

## Study area

Most samples were collected from a 10 x 1 m experimental channel that had been constructed in a bend of the Wainui Valley Stream seven months before sampling began. Additional samples were collected from riffles in unmodified regions of the stream where small stones were interspersed with larger stones up to 1 m in diameter.

The experimental channel was designed primarily to provide optimal conditions for *A. tillyardianum*. The bed of the experimental channel was covered with small stones 40-80 mm in diameter, and water flow was partly regulated to provide a current of 0.4-0.6 m $s^{-1}$. Colonization of the channel was through natural processes and, by the time sampling commenced, most species were present at densities comparable to those in riffle areas in unmodified parts of the stream.

## Methods

### *Sampling*

The sampling unit employed was a stone. In sampling, a stone was gripped and lifted through 30-150 mm of water, then placed in a plastic pot. Few animals were lost when sampling, as most species were firmly attached or closely applied to the rock surface. Further, the stones were slightly tilted as they were lifted so that the undersurface was directed downstream out of the full force of the current. In the conditions found in the Wainui Valley Stream, this method of sampling was believed to be more accurate than using nets. All animals on a stone were preserved in

90% ethanol within 2-4 hours of collection, and later were sorted and counted in the laboratory with the aid of a dissecting microscope.

Thirty-two collections were made between March and November 1971 and, except for the months March and April when two samples were taken each month, these were made at weekly intervals. Fifteen to 18 samples were taken on each occasion. All sampling was carried out between 1400 and 1600 h New Zealand Standard Time.

*Gut Analyses*

Gut analyses were made on 134 *H. parumbripennis* and 25 *H. colonica* larvae. Digestive tracts were dissected out and gut contents were transferred to a slide using glycerine as a mountant. Food items were recorded as detritus (including diatoms), plant material and animals. Animals were identified to specific level in most cases, and their numbers were recorded. Individuals of *A. tillyardianum* were identified to instar on the basis of morphological characters (number of antennal segments, degree of development of anal and semicircular sclerites, presence or absence of an egg burster), or by measuring structures (length of mandible, length of maxillary palp) (Crosby, in press).

Larval instars of trichopterans were determined by measuring head capsule widths to the nearest 25 µm using an eyepiece graticule mounted in a dissecting microscope. Size ranges of each instar are given in Table 2. In cases where the size classes overlapped, the overall size of a specimen was also used as a criterion for instar placement. *H. parumbripennis* has five larval instars, whereas only four size classes could be distinguished for *H. colonica*. It is probable that no first instar larvae of *H. colonica* were collected.

TABLE 2. Head capsule widths (µm) of larval instars of the two Trichoptera species

| | INSTARS | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *Hydrobiosis parumbripennis* | 150-225 | 225-375 | 400-625 | 750-925 | 1275-1375 |
| *Hydropsyche colonica* | | 300-475 | 575-600 | 875-1075 | 1475-1800 |

## Life histories of the predators during the sampling period

Most instars of *H. parumbripennis* were found each month (Fig. 1). Pupae were first found in August, and reached their highest numbers in October. In the experimental channel, densities averaged about 200 larvae $m^{-2}$ until the end of May, but at the beginning of June, there was a sharp decrease in numbers to about 30 larvae $m^{-2}$. This may have been a result of higher water levels and occasional flooding washing away larvae or causing them to move to deeper levels in the stream bed. Numbers remained low until October, when there was an increase to about 80 larvae $m^{-2}$ due to an influx of first instar larvae. In May, July and November, samples were also taken from unmodified areas of the stream that appeared to be similar to the experimental channel. In each month, population estimates derived from the unmodified areas were very similar to those derived from the experimental channel.

Larvae of the net-building trichopteran, *H. colonica*, were present in low numbers in the experimental channel. Densities of about 20 larvae $m^{-2}$ occurred until June, about 10 larvae $m^{-2}$ from July to October, and none were found in November. The gradual decrease in numbers can be attributed at least in part to the fact that no early instars were present to recolonize areas after periods of flooding during the sampling period (early instars were not found until December and January). This contrasts with their estimated densities in the unmodified areas of the stream where they were about four times as abundant during the same periods.

## Life histories of the two main prey species during the sampling period

*A. tillyardianum* is a multivoltine species which lives on the tops and sides of stones. During the sampling period, all instars were present at each sampling date, and at least nine cohorts completed their life histories during the period of study (Crosby, in prep.). Until the end of May, larval densities in the experimental channel were about 10 000 larvae $m^{-2}$. Flooding at the beginning of June reduced the population to about 2 000 larvae $m^{-2}$, and it remained close to this level until September when numbers began to increase. By November, the population was estimated at about 25 000 larvae $m^{-2}$. These population size estimates corresponded closely to those calculated for unmodified areas of the stream. Throughout

TABLE 3. Results of gut contents analyses made on larvae of *Hydrobiosis parumbripennis* and *Hydropsyche colonica*, March - November 1971

| Instar | Number of larvae | FOOD CATEGORIES | | | | |
|---|---|---|---|---|---|---|
| | | Animals only | Animals + plant material or detritus | Plant material + detritus | Detritus only | Empty |
| *H. parumbripennis* | | | | | | |
| 1 | 64 | 3 | 1 | - | 42 | 18 |
| 2 | 32 | 9 | 6 | - | 13 | 4 |
| 3 | 29 | 19 | 3 | - | 4 | 3 |
| 4 | 5 | 5 | - | - | - | - |
| 5 | 4 | 4 | - | - | - | - |
| *H. colonica* | | | | | | |
| 2 | 6 | - | - | 2 | 4 | - |
| 3 | 2 | - | - | - | - | 2 |
| 4 | 6 | - | 4 | 2 | - | - |
| 5 | 11 | - | 6 | 4 | 1 | - |

the sampling period, all size classes of *A. tillyardianum* were available as prey (Fig. 1).

Most chironomid larvae in the experimental channel were found in crevices on the sides and bottoms of stones. Normally the larvae occupied thin, loosely-woven, silken shelters, and some large larvae were found in empty *A. tillyardianum* cocoons. Chironomid larvae were present in low numbers for much of the sampling period and, until September, were at densities of about 500 to 1 000 larvae $m^{-2}$. During October and November, there was a marked increase in density to about 26 000 larvae $m^{-2}$ as a new generation of first instar larvae colonized the experimental channel. Thus, for the major part of the sampling period, only larger sized larvae were available as prey to the Trichoptera.

## Results and discussion

### *Food of larvae*

Although both species of Trichoptera are common in New Zealand, little has been reported on their feeding habits. *H. colonica* was stated to be omnivorous by Glasgow (1934) and Frances (1971), who both remarked that the amount of animal food taken was small, and although no information was found for *H. parumbripennis*, McFarlane (1969) has mentioned that gut content analyses of New Zealand Hydrobiosinae "show that the larvae eat a variety of other larvae particularly those of mayflies, sandflies [= simuliids] and midges (Chironomids)".

In the Wainui Valley Stream early instars of *H. parumbripennis* were mainly detrital feeders, whereas later instars were exclusively carnivorous (Table 3). In contrast, late instar larvae of *H. colonica* were omnivorous, with about 50% containing animal remains. Because of the low numbers of earlier instars collected, it was not possible to determine if animals constituted part of the diet of these larvae (Table 3). Plant tissue was found only in *H. colonica* larvae.

*H. parumbripennis* showed no sharp transition from a detrital to a carnivorous diet, but instead there was a gradual increase in number of animal food items in each successive instar until they were the exclusive foods of fourth and fifth instar larvae (Table 3).

Changes of diet between instars have been noted in relatively few studies of caddisfly feeding. Thut (1969) found that about 15% of the diet of second and third instars of the North American rhyacophilid, *Rhyacophila arnaudi* Denning, was detritus compared with only 4-5% in fourth and fifth instars, and Winterbourn (1971) found that in Marion Lake, British Columbia, the phryganeid *Banksiola crotchi* Banks fed mainly on filamentous algae in instars 2 and 3, animal prey became an important part of the diet in instar 4, and it predominated in instar 5.

### *Food of predaceous larvae*

The occurrence of different animal groups in larvae of *H. parumbripennis* and *H. colonica* which contained animal fragments is given in Table 4. The main animal prey of *H. parumbripennis* were larvae of *A. tillyardianum*, chironomids and *Deleatidium* sp. Also recorded were a few specimens of *Pycnocentrodes aureola* (McLachlan), *Tasiocera* sp., *H. parumbripennis* as well as unidentifiable remains. *A. tillyardianum* and chironomids were the most frequently encountered prey (Table 4). The first three instars of *H. parumbripennis* appeared to select chironomids rather than simuliids as prey whereas the converse was true for the final two instars. This was probably because chironomids were generally the smaller prey during the study period, and therefore may have been more easily ingested by the smaller caddis larvae.

Although *H. colonica* took fewer prey than *H. parumbripennis*, about as many prey groups were found (Table 4). *A. tillyardianum* was the main prey species recorded, and others were chironomids, *Neocurupira chiltoni* (Campbell), *H. colonica*, a species of hydrachnid and unidentifiable remains. Chironomids were less important in the diet of *H. colonica* than *H. parumbripennis* probably because they were present in low numbers in the experimental channel at the times *H. colonica* were found.

### *Number and size of prey taken*

In *H. parumbripennis*, the size and mean number of prey found per individual increased with each instar (Table 5). First to fourth instar larvae of *A. tillyardianum* (body lengths 0.6-1.3 mm) were taken mainly by second and third instar larvae of *H. parumbripennis*, whereas instars 5 to 8 (1.8-4.2 mm long) were taken only by fourth and fifth instar caddis. Most chironomids taken were first or second instars of the spring

TABLE 4. Occurrence of animal species in the guts of larvae of *Hydrobiosis parumbripennis* and *Hydropsyche colonica*

| | *H. parumbripennis*, instars | | | | | | | *H. colonica*, instars | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Total | % total | 2 | 3 | 4 | 5 | Total | % total |
| No. larvae examined | 64 | 32 | 29 | 5 | 4 | 134 | | 6 | 2 | 6 | 11 | 25 | |
| No. larvae with animal prey | 4 | 15 | 22 | 5 | 4 | 50 | | - | - | 4 | 6 | 10 | |
| Number with | | | | | | | | | | | | | |
| *Austrosimulium tillyardianum* | - | 5 | 8 | 3 | 3 | 19 | 38 | - | - | 3 | 4 | 7 | 70 |
| Chironomidae | 4 | 8 | 9 | 1 | 1 | 23 | 46 | - | - | - | 1 | 1 | 10 |
| *Deleatidium* sp. | - | 1 | 3 | 2 | 1 | 7 | 14 | - | - | - | - | - | - |
| Others | - | 1 | 11 | - | 1 | 13 | 26 | - | - | 1 | 4 | 5 | 50 |
| No. prey groups found | 1 | 4 | 7 | 3 | 4 | | | - | - | 2 | 6 | | |
| % larvae with *A. tillyardianum* | - | 33 | 36 | 60 | 75 | | | - | - | 75 | 67 | | |
| % larvae with Chironomidae | 100 | 60 | 41 | 20 | 25 | | | - | - | - | 25 | | |

TABLE 5. Numbers of prey in the guts of *Hydrobiosis parumbripennis* and *Hydropsyche colonica*, and the number of individuals containing the prey

| | *H. parumbripennis*, instars | | | | | | No. individuals in which occurring | *H. colonica*, instars | | | No. individuals in which occurring |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Total | | 4 | 5 | Total | |
| *Austrosimulium tillyardianum* | | | | | | | | | | | |
| Instar 1 | - | 17 | 4 | 3 | - | 24 | 6 | - | - | - | - |
| Instar 2 | - | 4 | 8 | - | - | 12 | 7 | - | - | - | - |
| Instar 3 | - | - | 4 | - | - | 4 | 3 | 1 | - | 1 | 1 |
| Instar 4 | - | 2 | 6 | - | 3 | 11 | 8 | 2 | - | 2 | 1 |
| Instar 5 | - | - | - | 1 | 2 | 3 | 2 | - | 2 | 2 | 2 |
| Instar 6 | - | - | - | 2 | 4 | 6 | 2 | 1 | - | 1 | 1 |
| Instar 7 | - | - | - | - | 5 | 5 | 3 | - | - | - | - |
| Instar 8 | - | - | - | - | 1 | 1 | 1 | - | 2 | 2 | 1 |
| Instar 9 | - | - | - | - | - | - | - | - | 1 | 1 | 1 |
| Pupa | - | - | 2 | - | 1 | 3 | 3 | - | - | - | - |
| Total | - | 23 | 24 | 6 | 16 | 69 | 19 | 4 | 5 | 9 | 7 |
| Chironomidae | 4 | 16 | 49 | 14 | 2 | 85 | 24 | - | 1 | 1 | 1 |
| *Deleatidium* sp. | - | 1 | 3 | 2 | 1 | 7 | 7 | - | - | - | - |
| Others | - | 1 | 11 | - | 1 | 13 | 13 | 1 | 4 | 5 | 5 |
| TOTAL | 4 | 41 | 87 | 22 | 20 | 174 | 50 | 5 | 10 | 15 | 10 |
| Mean no. prey per individual | 1.0 | 2.7 | 4.0 | 4.4 | 5.0 | 3.5 | | 1.25 | 1.7 | 1.5 | |

generation, and most were taken by early instars of *H. parumbripennis*.

Although few animals were found in guts of *H. colonica*, it appeared that the mean number of prey per individual was about three times lower than that for *H. parumbripennis* (Table 5). It was not possible to assess whether different instars selected different sized prey.

In other studies on Trichoptera, Winterbourn (1971) found that the mean size and size range of chironomid larvae taken by *B. crotchi* increased with each instar, Tachet (1965b) mentioned that *Polycentropus flavomaculatus* (Pictet) appeared to consume smaller prey in its earlier instars, and a similar predator-prey size relationship was reported by Haage (1970) for *Phryganea grandis* Linnaeus.

The mean number of animal prey found in the guts of *H. parumbripennis* was about 3.5 (Table 5). This compares with means of 2.2 and a little less than 2.0 obtained for *Polycentropus flavomaculatus* by Tachet (1965a) and Jones (1950) respectively. For *Rhyacophila dorsalis* (Curtis) from England, the mean number of simuliid larvae and pupae found in the guts was cautiously estimated as 2-3 by Jones (1950).

In *H. parumbripennis*, the number of prey found in each individual increased with larval size (Table 5), in contrast to the situation found in *P. flavomaculatus* in which the smaller larvae contained more prey per individual (2.6-2.7) than larger ones (1.6-1.7) (Tachet, 1965b). The number of prey found in the later instars of *P. flavomaculatus* is very similar to that found for the final instars of *H. colonica*, however, which may be a reflection of the fact that both species rely to some extent on prey being captured by nets, as opposed to the active predatory behaviour of *H. parumbripennis*.

### *Forage ratios and prey availability*

Comparisons between the relative abundance of animals collected during the sampling period and the abundance of these animals in the gut contents of *H. parumbripennis* were investigated using a forage ratio first formulated by Hess & Swartz (1940), quoted by Tachet (1965a).

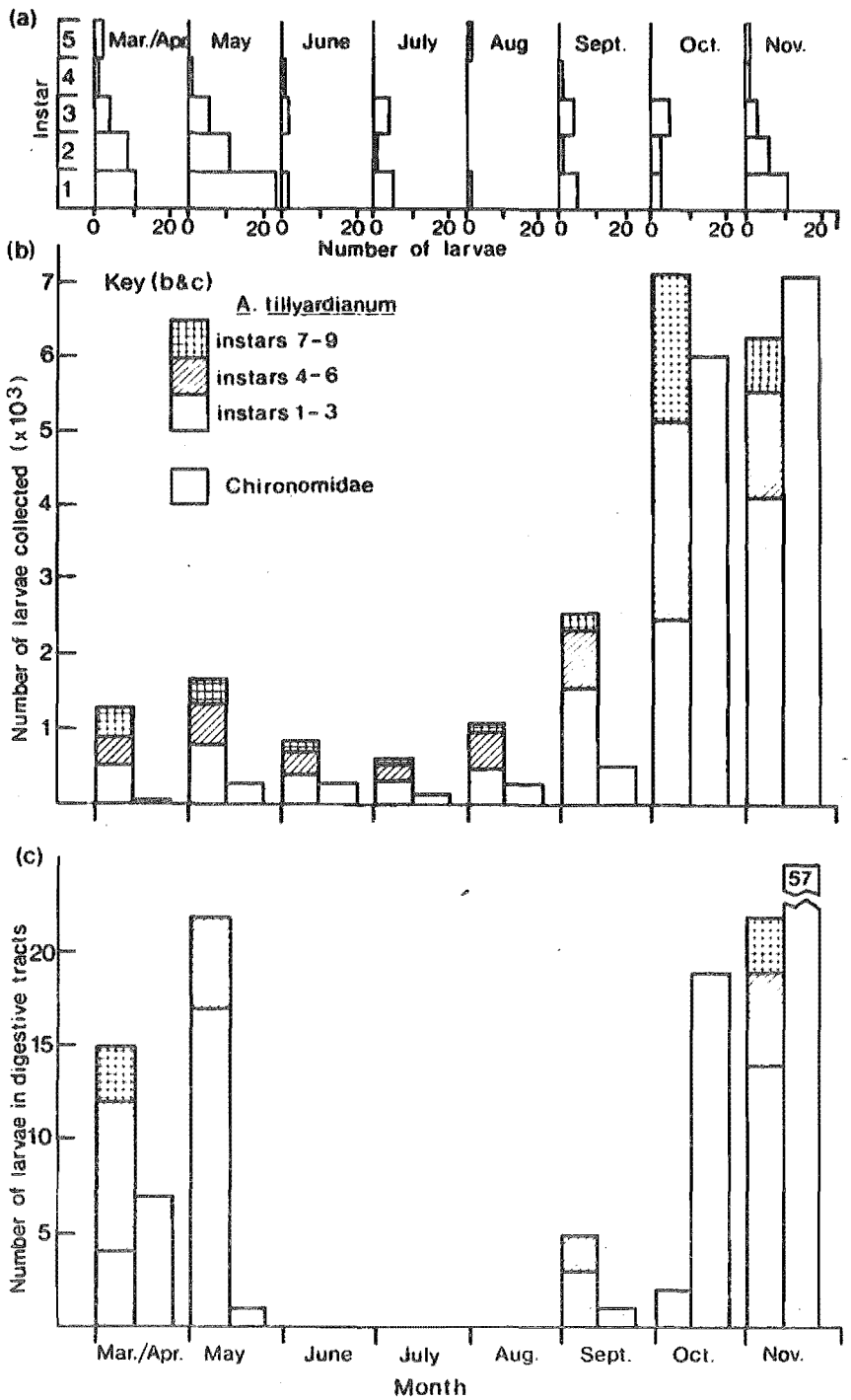


Fig. 1. Numbers of (a) *Hydrobiosis parumbripennis*, (b) *Austrosimulium tillyardianum* and Chironomidae collected from the stream each month, and (c) *A. tillyardianum* and Chironomidae found in the digestive tracts of *H. parumbripennis* each month.

$$\text{Forage ratio} = \frac{\%\text{ of prey } x \text{ in the digestive tract of predator } y}{\%\text{ of prey } x \text{ in the fauna}}$$

Over the entire study period the forage ratios for *A. tillyardianum*, Chironomidae and *Deleatidium* sp. were 0.9, 1.7 and 3.8 respectively, which indicates that simuliids were being utilized as prey in proportion to their abundance in the fauna, whereas the others were being selected preferentially as food items. It is interesting to note that during October and November when *A. tillyardianum* and chironomid larvae were present in equally high numbers, chironomids appeared to have been preyed upon preferentially (Fig. 1) presumably because they were the smaller sized prey. The comparatively high forage ratio obtained for *Deleatidium* sp. may be related to the fact that mayfly larvae are normally found on the same parts of stones as *H. parumbripennis*, and therefore may be more likely to be encountered. A forage ratio of 0.2 was obtained for *P. aureola* indicating that this species was avoided as a food item. This is almost certainly related to its possession of a protective case. The same reason may also apply to larvae of the trichopterans *Helicopsyche* sp. and *Pycnocentria evecta* McLachlan and the gastropod *Potamopyrgus antipodarum* (Gray) which were not utilized as food items.

Although simuliids were eaten in about the same proportion as their abundance, the relative proportions of different sized larvae eaten appeared to differ from the proportions present in the stream in some months. In May, September and October, for example, no large instars were found in the gut contents of *H. parumbripennis* although these instars were available as prey (Fig. 1). This was probably because at these times few large *H. parumbripennis* capable of eating large prey were present in the stream.

### *Importance of* H. parumbripennis *as a predator of* A. tillyardianum

Trichoptera have been recorded as predators of Simuliidae throughout the world and detailed lists of these instances have been given by Jenkins (1964) and Burton & McRae (1972). Examination of the digestive tracts of trichopterans in some studies has revealed that simuliids were found in only about 10% of large larvae. For example, in Ghana of the carnivorous Trichoptera collected from breeding grounds of *Simulium (Edwardsellum)*

*dammosum* Theobald, 5 out of 50 larvae of *Hydropsyche* sp., and 3 out of 25 *Cheumatopsyche* sp. larvae contained simuliid larvae (Burton & McRae, 1972). One out of the 9 specimens of the European species *Rhyacophila dorsalis* examined by Slack (1936) contained simuliid larvae, although Jones (1950) found that all 12 specimens of this species that he examined contained simuliid larvae or pupae fragments. Other studies only report that simuliids were found to be present in the gut contents (Badcock, 1949; Peterson & Davies, 1960, and others). In several of these studies, simuliids were present in high numbers with the trichopterans.

In this study, 19 out of the 134 larvae of *H. parumbripennis* examined contained *A. tillyardianum* larvae (Table 4), and when the total numbers of *A. tillyardianum* in the Wainui Valley Stream are compared with the number taken as prey by *H. parumbripennis* (Fig. 1, Table 5), it is evident that the predation rate is low. Even if the numbers of *H. parumbripennis* have been underestimated by the sampling method which only collected those larvae living on the surface stones of the stream bed, it is probable that predation by *H. parumbripennis* has little effect on the size of the simuliid population compared with other factors such as intraspecific competition for attachment sites and flooding.

Even though predation by *H. parumbripennis* was low, it appeared to be the most important invertebrate predator of *A. tillyardianum*, as apart from *H. colonica* no other carnivorous insects were found in the Wainui Valley Stream. Fish predation appeared to be unimportant also, as of the 10 long-finned eels, *Anguilla dieffenbachii* Gray, 14 red-finned bullies, *Gobiomorphus huttoni* (Ogilby), and 3 galaxiids, *Galaxias fasciatus* Gray, collected by electric fishing in March 1971, only two *G. huttoni* contained *A. tillyardianum*. Further, the number of simuliids present was only 5, which constituted only 2.1% of the total organisms eaten by the 14 *G. huttoni*. Other studies on the diets of New Zealand freshwater fishes have also shown that simuliids are unimportant components of their diets (Allen, 1951; McDowall, 1965, 1968). This contrasts with their reported importance in the diet of young stages of some fish elsewhere (Dimick & Mote, 1934; Frost, 1939; Allen, 1941; Maitland & Penney, 1967; Power, 1969, and others).

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Key words: New Zealand, food of Trichoptera, experimental channel, Trichoptera, Rhyacophilidae, *Hydrobiosis parumbripennis*, Hydropsychidae, *Hydropsyche colonica*, Simuliidae, *Austrosimulium tillyardianum*, Chironomidae, predation rate, prey size selection.

Paper 5

# Trichomycetes (Harpellales) of New Zealand *Austrosimulium* larvae (Diptera : Simuliidae)

Accepted for publication in: *Journal of Natural History*

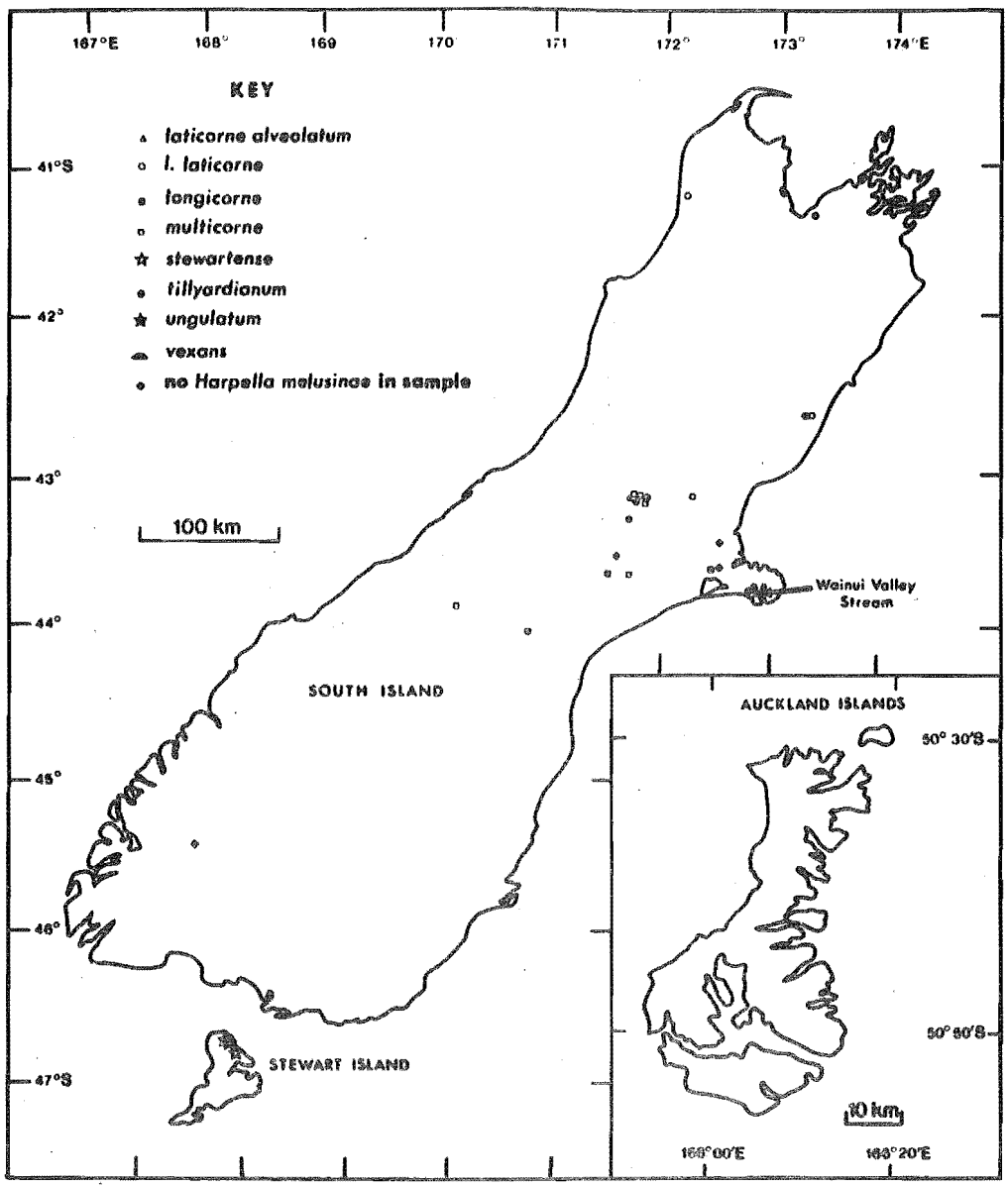
## Introduction

With the exception of the ectozoic genus *Amoebidium*, the Trichomycetes are a class of fungi found living as commensals in the guts of arthropods (Manier, 1969; Lichtwardt, 1973). Their distribution is apparently worldwide, although detailed studies have so far been carried out only in France and North America. This paper records the occurrence of three species of Trichomycetes found in the digestive tracts of New Zealand *Austrosimulium (Austrosimulium)* Tonnoir larvae: *Harpella melusinae* Léger & Duboscq, *Smittium* sp. and another unidentified genistellacean. All three are new Southern Hemisphere records. Also given are some details of the life history and abundance of the commonest species, *H. melusinae*.

The order Harpellales is divided into two families; the Harpellaceae, containing species with unbranched thalli which attach to the peritrophic membrane lining the midgut of dipteran larvae, and the Genistellaceae, containing species whose thalli are branched and attached to the hindgut lining in several orders of immature aquatic insects (Manier, 1969; Lichtwardt, 1972). *Harpella melusinae* is the only harpellacean found in simuliid larvae, the other species of the family being found in the midguts of larval Ceratopogonidae and Chironomidae. Amongst the Genistellaceae, eight species from five genera are known to inhabit the hindgut of simuliid larvae (Manier, 1969; Lichtwardt, 1972), and two species from two genera have been found in the hindgut of *Austrosimulium (Austrosimulium)* larvae. These are *Smittium* sp. and an unidentified genistellacean. The only other trichomycete found in the hindgut of a simuliid larva, *Paramoebidium chattoni* Duboscq, Léger & Tuzet (order Amoebidiales) has not been found in *Austrosimulium (Austrosimulium)* although it is common in several genera in other parts of the world (Moss, 1970; Lichtwardt, 1972).

## Midgut trichomycete, *Harpella melusinae*

Since the first description of *Harpella melusinae* by Léger & Duboscq (1929), its occurrence in France has been further documented by Tuzet & Manier (1955) and Manier (1969). It is also common in many Northern Hemisphere localities including England (Moss, 1970), U.S.A. (Lichtwardt, 1967, 1972), Canada (Frost & Manier, 1971) and Japan (Lichtwardt, 1967) and, altogether, has been recorded in 17 species of Northern Hemisphere Simuliidae. *H. melusinae* is also common in New Zealand where I have found it in seven species of *Austrosimulium* (fig.). Further, infections have




Localities in New Zealand where *Austrosimulium* larvae have been examined for the presence of *Harpella melusinae*; positive records are shown by the *Austrosimulium* host species. One positive record for *A. (A.) tillyardianum* from the Wainuiomata River, North Island (41° 16'S, 174° 57'E) is not shown. Nomenclature for the *Austrosimulium* species follows that of Dumbleton (1973).

been detected in all samples containing over five *Austrosimulium* larvae.

Moss (1970) has recently described for *H. melusinae* the immature thalli and the cytological changes that occur during the formation of asexual trichospores by mature thalli. The fine structure of thalli and trichospores was investigated in an electron microscope study by Reichle & Lichtwardt (1972). Earlier accounts on trichospores have also been given by Léger & Gauthier (1935) and Lichtwardt (1967). These aspects of the life history of *H. melusinae* will therefore not be considered in this paper.

## Observations on reproduction

Sexual reproduction of *H. melusinae* is not common, and consists of scalariform conjugation of thalli followed by the formation of pointed bipolar zygospores (Lichtwardt, 1967). Conjugation among thalli inhabiting *A. (A.) tillyardianum* larvae was observed in only four out of about 200 larvae examined, and no zygospores were found. The lack of zygospores is not surprising, however, since they have been recorded previously in only two specimens of the North American species *Simulium (Psilozia) vittatum* Zetterstedt (Lichtwardt, 1967).

The conjugation rate of about 2% obtained in *A. (A.) tillyardianum* appears to be considerably lower than that recorded for North American simuliids. Although he gave no comparative figures, Lichtwardt (1967) stated that "Conjugation of thalli is a fairly common phenomenom in *Harpella*", which suggests that the midgut environment provided by *Austrosimulium* may be slightly different from that found in other simuliid genera. Another possibility is that since all larval instars of some *Austrosimulium* species can be found at all times of the year, a 'resting' zygospore is not necessary to maintain populations of *H. melusinae* in New Zealand.

The caudal filaments of the asexual trichospores probably become entangled in debris on the substratum, or in the cephalic fans of neighbouring larvae, upon their release (Lichtwardt, 1967). Since most simuliid larvae inhabit water flowing at 0.3-2.0 m $s^{-1}$ (Phillipson, 1956; author's observations) where spores could easily be washed away, an efficient transmission mechanism is necessary to maintain population numbers in a particular area. That this system of trichospore transmission is very successful is indicated by the high infection rates found in many *Austrosimulium* populations inhabiting coastal streams less than 3 km long, and

the very high numbers of *H. melusinae* found in many *Austrosimulium* larvae.

## Abundance and occurrence in different species

*H. melusinae* may be particularly abundant in *A. (A.) tillyardianum* larvae, and 90% to 100% infections of larvae are not unusual when larval densities are greater than 10 000 $m^{-2}$. Within an individual larva, the number of thalli may also be high; for example, in July 1969 and October 1970, ninth instar larvae of *A. (A.) tillyardianum* from the Wainui Valley Stream, Canterbury, contained an average of almost 60 thalli per larva, with some larvae containing about 120 (table). Even though infections are heavy, they have no apparent effect on the host larvae.

Few reports on infection rates in other simuliids are available for comparison. Moss (1970) reported that some larvae of *Simulium (Wilhelmia) equinum* Linnaeus from England contained up to 20 thalli, and that about 75% of larvae examined, whereas Williams & Lichtwardt (1971) stated that *H. melusinae* "was found attached to the peritrophic membrane of most larvae" of *Simulium (Psilozia) vittatum* and *Simulium (Simulium) venustum* Say from the U.S.A., but they did not give any figures on infection rates.

*H. melusinae* has not been found to be so abundant in other *Austrosimulium* species, however. Although high infection rates of up to 75% have been found in *A. (A.) multicorne* Tonnoir, *A. (A.) stewartense* Dumbleton and *A. (A.) vexans* (Mik) larvae from some localities, the number of thalli per larva has seldom exceeded 20. Furthermore, no signs of conjugation have been found in these species.

It was also of considerable interest to find that *H. melusinae* was common in *A. (A.) vexans*, a simuliid occurring on the Auckland Islands, an island group about 400 km south of New Zealand. Its presence in a small island population indicates both the initial dispersal capacity of *H. melusinae* and the present efficiency of trichospore transmission from host to host. It is also good evidence that *H. melusinae* is a cosmopolitan species, and should be found in other parts of the world when it is looked for.

Despite the high numbers of *H. melusinae* found in larvae of *Austrosimulium*, it has not been found in any other associated invertebrates. For example, in October 1970 the final instar larvae of *A. (A.) tillyardianum*

from the Wainui Valley Stream were 100% infected with *H. melusinae*, but examination of 10 specimens of each of the following seven species did not reveal the presence of *H. melusinae*: a blephariceríd, *Neocurupira chiltoni* (Campbell); two chironomid species; two trichopteran species, *Hydrobiosis parumbripennis* McFarlane and *Hydropsyche colonica* McLachlan; an ephemeropteran, *Deleatidium* sp.; and a plecopteran, *Zelandoperla maculata* (Hare). Such a result is further evidence of the host specificity of *H. melusinae* for simuliid larvae.

## Instar at which infection occurs

Since the population of *A. (A.) tillyardianum* in the Wainui Valley Stream was heavily infected with *H. melusinae* in October 1970, the opportunity was taken to examine larvae of all instars to discover the earliest instar at which *H. melusinae* was able to infect and produce trichospores. The number of larvae of each instar examined and the number infected is given in the table.

*Number of larvae of* Austrosimulium (Austrosimulium) tillyardianum *of each instar examined for the presence of* Harpella melusinae, *the number of larvae infected and the maximum number of thalli found in each instar. A.* (A.) tillyardianum *collected from the Wainui Valley Stream, October 1970.*

| Instar | Number of larvae examined | Number of larvae infected | Maximum number of thalli observed |
|---|---|---|---|
| 1 | 6 | 0 | 0 |
| 2 | 9 | 4 | 1 |
| 3 | 10 | 8 | 2 |
| 4 | 10 | 10 | 5 |
| 5 | 5 | 5 | 12 |
| 6 | 5 | 5 | 35 |
| 7 | 5 | 5 | *ca.* 80 |
| 8 | 5 | 5 | *ca.* 100 |
| 9 | 5 | 5 | *ca.* 120 |

No first instar larvae (0.52 mm long) were infected, probably because infective trichospores are too large to be ingested by these small larvae. About 50% of second instar larvae (0.75 mm long) were infected, and in these cases, only thallus producing a single trichospore was present. 80% of third instar larvae (0.99 mm long) were infected, and usually two

thalli producing one or two trichospores were present. By the fourth instar (1.32 mm long) all larvae were infected with *H. melusinae*, each larva having two to five thalli producing one to three trichospores. The number of thalli in each larva hereafter increased with instar, reaching an average of about 60 thalli each producing one to five trichospores by the ninth and final instar.

In *A. (A.) tillyardianum*, the peritrophic membrane normally takes at least two or three days to travel the length of the midgut (author's observations), and, in this time, *H. melusinae* completes its life history. Since *H. melusinae* is capable of producing trichospores from the second instar onwards, this indicates that the gut contents of *A. (A.) tillyardianum* larvae take as long to travel through the midgut of a second as a ninth instar larva. This is in spite of the fact that the midgut of a ninth instar larva is about 10 times the length of a second instar larva. Presumably, because the food collected in all instars is similar (microseston of Maciolek & Tunzi (1968)), the same length of time is required for the food to be digested in each instar.

## Hindgut Trichomycetes

Seventeen larvae of the Wainui Valley Stream population of *A. (A.) tillyardianum* were examined for the presence of hindgut trichomycetes. Two species were found, an unidentified genistellacean which occurred in five larvae, and *Smittium* sp. which occurred in two. No more than two thalli per larva were found for the unidentified genistellacean, and only three were producing trichospores. The *Smittium* sp. found resembles the widespread species *S. simulii* Lichtwardt, which has been recorded from North America and Europe (Lichtwardt, 1964; Manier, 1969). The three thalli of *Smittium* sp. were all producing collared trichospores characteristic of the genus.

Until further material is examined, the specific identity of the *Smittium* and the generic identity of the genistellacean will remain uncertain. However, it is likely that both these taxa will be found to be more widespread when simuliid larvae from other localities and different species are examined, and it is very likely that other taxa of Trichomycetes will also be discovered.

## Summary

Three species of Trichomycetes were found in New Zealand *Austrosimulium (Austrosimulium)* Tonnoir larvae; *Harpella melusinae* Léger & Duboscq, *Smittium* sp. and an unidentified genistellacean. All three are new Southern Hemisphere records. The commonest species, *H. melusinae*, was found in seven species of *Austrosimulium* being particularly abundant in *A. (A.) tillyardianum* Dumbleton. In a sample of *A. (A.) tillyardianum* examined from the Wainui Valley Stream, Canterbury, it was found that *H. melusinae* was able to infect and produce trichospores in second instar larvae, and that the number of thalli and trichospores per larva increased in each subsequent instar.

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Paper 6

# Wing and haltere venation in larvae of the *Austrosimulium (Austrosimulium) australense* group from New Zealand (Diptera : Simuliidae)

Accepted for publication in: *Journal of Entomology (B)*

## INTRODUCTION

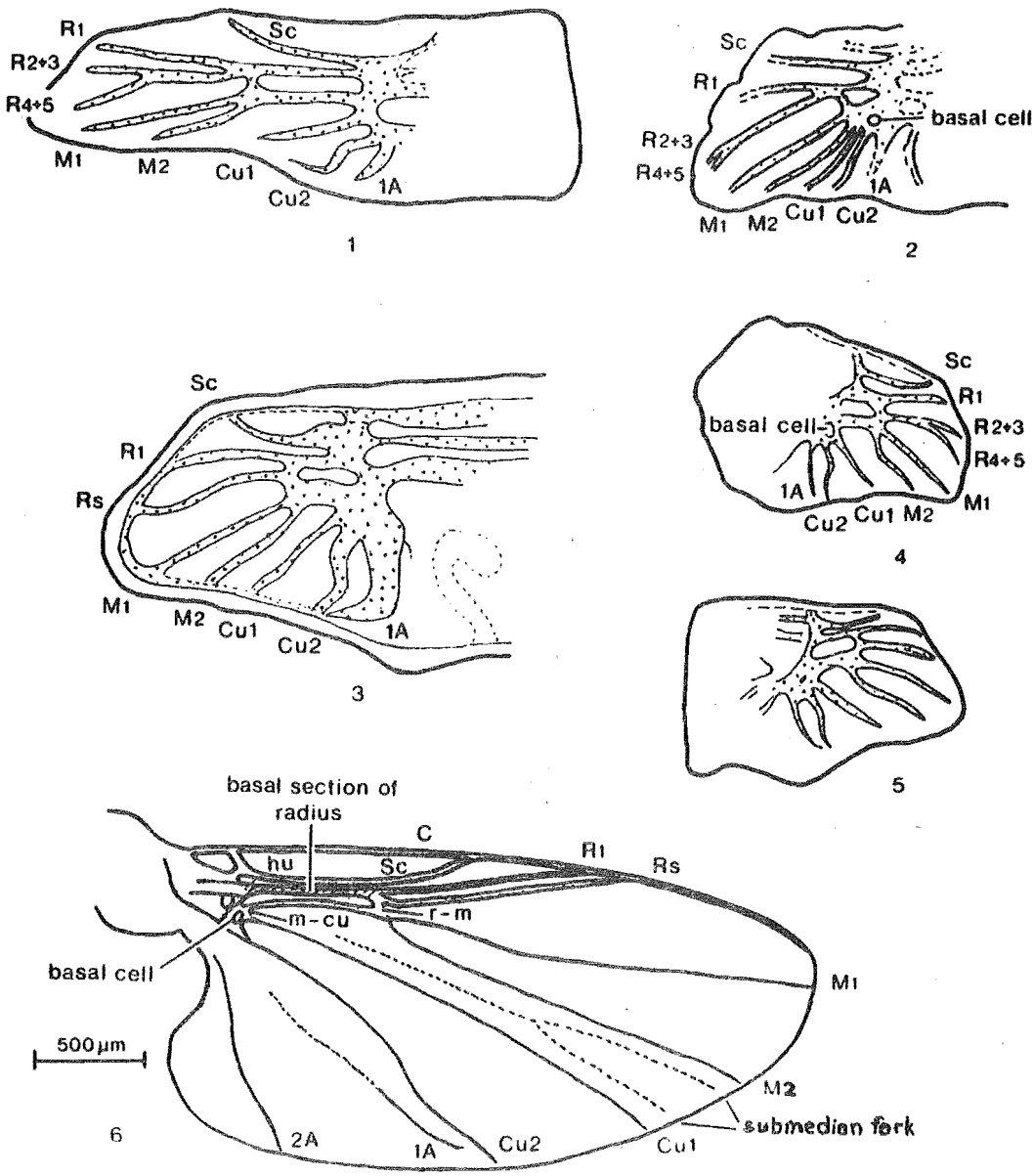
Often the development of wing venation of an insect can be traced from the wing bud of a larva to its final form in an adult. Changes in the venation pattern may occur during the course of this development, and sometimes it is possible to relate the venation changes to either the proposed homologies of the veins (Imms, 1964; Wisely, 1965) or to the supposed phylogeny of a group (Edwards, 1934; Smart, 1945).

The relationship between the larval and adult venation pattern is the result of the manner in which veins develop. In the first part of the process, blood-filled lacunae appear in the developing wing bud of a larva. Differential sclerotization of the lacunal integument involved in the adult venation then occurs, and finally trachea usually grow into the lacunal network. The sclerotized network so formed is the basis of the adult venation (Imms, 1964).

In the Simuliidae any venation pattern seen in wing or haltere buds of final instar larvae is that formed by the lacunae only, since sclerotization of the lacunal integument takes place in the pupa. The name larval wing venation has been given to the wing bud lacunal pattern (Edwards, 1934), and the name "larval haltere venation" is proposed for the haltere bud lacunal pattern.

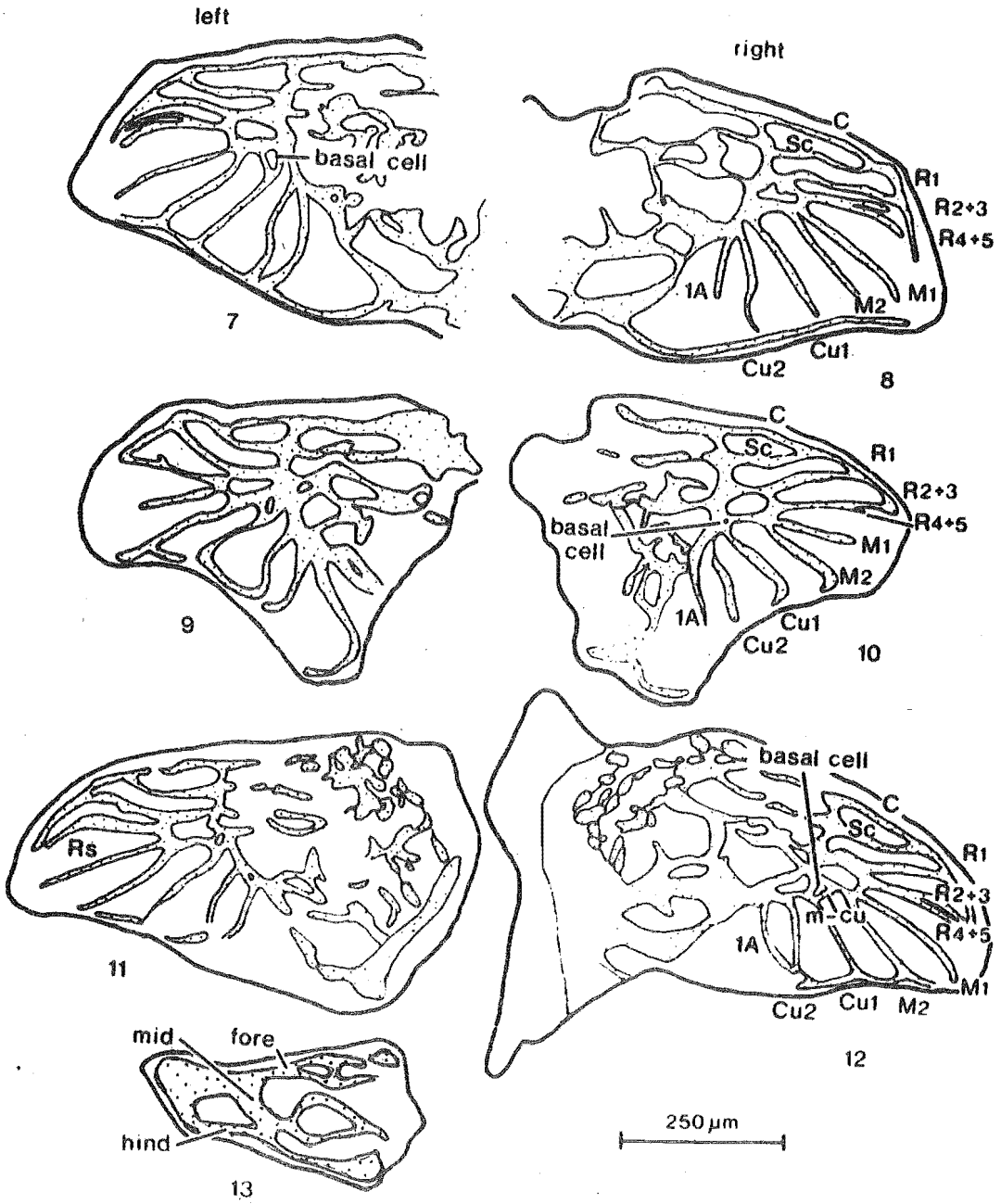
## OCCURRENCE OF LARVAL VENATION IN SIMULIIDAE

Because the blood of Simuliidae is transparent, larval venation normally is not visible in final instar larvae. Larval wing venation appears to have been recorded in only four species, (*Prosimulium (Helodon) ferrugineum* (Wahlberg), *Metacnephia pallipes* (Fries) and *Simulium (Odagmia) ornatum* Meigen from the Palaearctic region (figs. 1-3) (Rubzov, 1940:47), and *Simulium (Simulium) feuerborni* Edwards from Java (figs. 4 and 5) (Edwards, 1934:132)), and no larval haltere venation has been reported previously. Edwards, after his description of *S. feuerborni*, remarked that "before examining this species I had never observed any definite structure in the imaginal disc of the wing. However, in nearly all the full-grown larvae of *S. feuerborni* examined the larval wing shows the venation so clearly as to appear almost like a minute replica of the adult wing."




**Figs. 1-6.** (1-3) Larval wing venation, after Rubzov (1940): (1) ***Prosimulium ferrugineum***; (2) *Metacnephia pallipes*; (3) *Simulium ornatum*. (4,5) *Simulium feuerborni*, larval wing venation of wing buds dissected from two different larvae (after Edwards, 1934). (6) *Austrosimulium tillyardianum*, venation of adult wing.

**Abbreviations of veins:** *1A* and *2A* - anal; *C* - costa; *Cu1* and *Cu2* - cubitus; *hu* - humeral; $M_1$ and $M_2$ - media; *m-cu* - media cubitus crossvein; $R_1$ - radius; $R_{2+3}$ and $R_{4+5}$ - branches of radial sector; *r-m* - radius media crossvein; *Rs* - radial sector; *Sc* - subcosta.




**Figs. 7-13.** (7,8) *Austrosimulium laticorne*, wing venation of an individual larva : (7) left wing bud, basal cell present; (8) right wing bud, basal cell absent. (9,10) *Austrosimulium multicorne*, wing venation of an individual larva : (9) left wing bud; (10) right wing bud. (11,12) *Austrosimulium tillyardianum*, venation of an individual larva : (11) left wing bud, unforked radial sector; (12) right wing bud, partially forked radial sector. (13) *Austrosimulium laticorne*, larval haltere venation of left haltere bud; interconnected fore, mid and hind veins.

Examination of final instar larvae of *Austrosimulium (Austrosimulium)* spp. from New Zealand has shown that in the six species of the *australense* group studied (*australense* (Schiner), *laticorne* Tonnoir, *longicorne* Tonnoir, *multicorne* Tonnoir, *stewartense* Dumbleton and *tillyardianum* Dumbleton) the lacunae contain chromatocytes and a larval wing and haltere venation can be distinguished. Larvae of *A. ungulatum* Tonnoir and *A. vexans* (Mik) at the equivalent stage of development do not have pigmented lacunae; instead the lacunae are partially visible as light lines on a darker wing bud when viewed with a compound microscope. The *A. ungulatum* group condition appears to be representative of the condition found in other simuliid species where larval venation is not noticeable (Edwards, 1934).

Larval venation of the *A. australense* group is most clearly defined at the time when the developing pupal respiratory filaments start to change in colour from white to brown. According to Hinton (1958) this occurs soon after the larva-pupa apolysis, but still several days before the pupa splits the larval cuticle. Once the respiratory filaments turn black, the venation pattern becomes difficult to recognize because the wing and haltere buds increase in size and the chromatocytes become separated from each other or disappear.

## COMPARISON OF LARVAL AND ADULT WING VENATION

All species of the *A. australense* group studies had a larval wing venation that corresponded to that of the adult as far as the presence of the main veins was concerned (figs. 6-12). However, two important differences in the patterns could be seen upon closer examination, both of which were noted by Edwards (1934) in *S. feuerborni*. Firstly, a submedian fork was present in the adult venation, but was not traceable in the larval wing venation, and secondly the radial sector vein, *Rs*, was simple in the adult but forked in the larval venation.

### *Submedian fork*

The submedian fork is a characteristic feature of the simuliid wing, and may be homologous with the simple fold usually found in the same position among Chironomidae and Scatopsinae (Edwards, 1931). The submedian fork does not disappear when the wing is macerated with 10 per cent KOH, unlike the secondary venation of the Blephariceridae which is the result of pupal folding, and this led Edwards (1931) to suggest that the submedian fork was the

vestige of a true vein, perhaps homologous with the posterior media, $M_{3+4}$. When he discovered that there was no sign of a submedian fork in the larval venation of *S. feuerborni*, however, he reconsidered his former view and concluded that probably the fork was not a vein as first supposed (Edwards, 1934). No submedian fork was seen in the three Palaearctic species examined by Rubzov (1940) and he also concluded that it was not a true vein. Likewise, the lack of a submedian fork in larval venation of the *A. australense* group substantiates the later view of Edwards and that of Rubzov.

Further evidence is provided by the appearance of the submedian fork after the adult wing has been macerated in KOH, a process which causes upper and lower membranes of a wing to separate, allowing each membrane to be examined individually. Wing veins normally are of two types; convex veins which are more strongly marked or bear setae on the upper membrane, and concave veins which are more strongly marked or bear setae on the lower membrane. The submedian fork does not appear to fit into either group as it is almost equally well marked on both membranes (perhaps the lower membrane is slightly better marked) as shown by the alignment of the microtrichia. The rest of the venation consists of either convex or concave veins. This finding is contrary to that of Edwards (1931) who found that the submedian fork was distinct only on the lower membrane in the simuliids he studied, a finding from which he concluded at that time that the fork could be homologous with the concave posterior media of other Diptera.

### *Radial sector vein*, Rs

#### Specific and individual differences in the presence of the *Rs* fork

In *Austrosimulium* there appears to be differences between species in the clarity with which branching of the radial sector vein is shown (i.e. the $R_{2+3}$ and $R_{4+5}$ branches of the *Rs*). Only about 25 per cent of the *A. tillyardianum* larvae examined showed *Rs* forking, and in these the $R_{2+3}$ and $R_{4+5}$ branches were almost joined to each other. By contrast, all specimens of the *A. laticorne* larvae examined showed the forking clearly. The clarity with which *Rs* forking was shown in the other four species examined was intermediate between the above two species. In well developed larvae of all species it was difficult to judge if forking had occurred because the chromatocytes in the lacunae were beginning to separate from each other.

In individual larvae of the *Austrosimulium* species, the clarity of the larval wing venation often differed between the left and right wing buds. For example, *Rs* forking may have been evident on one side of the larva but not on the other. Such differences are illustrated for *laticorne, multicorne* and *tillyardianum* (figs. 7-12).

Phylogenetic implications for the origin of *Austrosimulium*

In many Prosimuliini the radial sector vein *Rs* is forked in the adult wing, but in Simuliini adults it is always simple. The presence of a simple *Rs* has been thought of as being a derived (apomorphic) character in simuliids, especially when considered in combination with other characters. Evidence from an examination of larval wing venation reinforces this opinion. Edwards (1934) found that larvae of the simuliine species *S. feuerborni* possessed a forked *Rs*, and therefore suggested a prosimuliine form as the ancestral group of the family.

The genus *Austrosimulium* is a member of the Simuliini (Crosskey, 1969), thus it was interesting to find that the species of the *A. australense* group investigated possessed a forked *Rs* in the larval wing (figs. 7-12). This finding is interpreted as further evidence that the simuliine line is derived from the prosimuliine line.

The correspondence of the larval venation pattern and subsequent adult venation pattern between *Austrosimulium* and *Simulium* also suggests that these genera have a common ancestry, which supports the common ancestry view advanced by Dumbleton (1963, 1964, 1973). It is less likely that *Austrosimulium* could have been derived from prosimuliine stock as a line separate to that of *Simulium*, and then through convergence come to resemble the *Simulium* line.

## LARVAL HALTERE VENATION

Species of the *A. australense* group investigated showed traces of a reduced venation in the larval haltere pad, although, as is normal in Diptera, there was no sign of venation in the adult halteres. Larval haltere venation has not been recorded previously for any simuliid species, nor apparently for any other Diptera.

Haltere venation in the *A. australense* group appears to consist of connected fore, mid and hind veins (fig. 13), but it is problematic whether standard venational names can be applied to them. The pattern of veins resembles an illustration in Imms (1964:596) of a mutant *Drosophila melanogaster* Meigen adult in which halteres were replaced by hind wings. On the basis of the correspondence between the larval wing venation and the resulting adult venation, the presence of veins in the larval haltere of *Austrosimulium* is considered to represent evidence that halteres of Diptera are highly modified hind wings.

Previous evidence for this view has been based upon their development from dorsal metathoracic imaginal buds (Imms, 1964), upon adult *D. melanogaster* mutants in which venation patterns could be recognized (Lindsley & Grell, 1968) or upon cultures derived from haltere discs (Gehring & Nöthiger, 1973). The occurrence of haltere venation in *Austrosimulium* larvae and its subsequent loss in adult halteres appears to be the first evidence to indicate that a reduced venation pattern may be a normal feature of haltere development in Diptera, and that haltere venation is not just a mutation phenomenon expressed in some adults of *D. melanogaster*.

## SUMMARY

The venation pattern found in the developing wing and haltere buds of species of the *Austrosimulium (Austrosimulium) australense* group of New Zealand Simuliidae is discussed. The larval wing venation and the resulting adult wing venation correspond in their main features. Two differences in the venation patterns are noted; firstly, in the larval wing venation there is no submedian fork characteristic of the adult wing, and secondly, the radial sector vein *Rs* is forked in the larval venation but is simple in the adult. The significance of these differences for indicating the proposed homologies of veins and the supposed phylogeny of the genus is commented upon. The presence of a venation pattern in the larval haltere is interpreted as further evidence that halteres of Diptera are highly modified hind wings.

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Paper 7

# DYAR'S RULE PREDATED BY BROOKS' RULE

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When enumerating the larval instars of an insect, it is often difficult to decide if an instar has been overlooked. A method to check if an instar series is complete has been to use Dyar's rule which states that there is a geometric increase in the size of a sclerotized structure with each instar (Dyar 1890). When the logarithm of a measurement is plotted against the appropriate instar number, a straight line should be obtained. If there is any marked deviation from a straight line, it may indicate that an instar has been missed at the point of the deviation (Wigglesworth 1965).

Dyar (1890) formulated this rule after rearing the larval stages of 28 species of Lepidoptera. He found 'that the widths of the head of a larva in its successive stages follows a regular geometrical progression, and if . . . . any deviation from the calculated progression is shown, it is evidence that an error has been committed or that the larva has behaved in an abnormal manner'.

In fact, such a progression between moults had been noted four years previously in Crustacea by Brooks (1886). Brooks measured a collection of similar looking erichthi larvae and was able to determine that they represented a series of the larval stages of *Lysiosquilla minuta* Brooks (Stomatopoda: Squillidae). He found that if the total 'length of the first stage be successively multiplied by five-fourths of itself, and this number by five-fourths of itself again, and so on' the series of numbers so computed corresponded to the total lengths of the series of larval stages measured. An exception was that the computed series indicated that the penultimate larval stage was missing from the measured series.

The numerical relationship found by Brooks for Crustacea must be

considered synonymous with the geometric growth rule proposed by Dyar for Lepidoptera. Further, it should be noted that both authors indicated that their relationship could be used to decide if a larval stage had been missed from a measured series. It follows then that the name given to the geometric relationship and to its use for detecting missing stages should be credited to Brooks and not to Dyar. The priority of Brooks' name for the rule has been recorded before (Teissier 1960) but the priority was documented in a book devoted to Crustacean physiology, and therefore has been overlooked by entomologists, as well as by abstracting journals.

The rule itself, as proposed by Brooks, was rediscovered only recently (Teissier 1960), and the main reason that it has been overlooked for so long is because it was published in a monograph concerned with the taxonomy of a collection of Stomatopoda which only specialists of the group would be likely to consult. Another reason is that once the appropriate paragraphs have been located, the text describing the rule is difficult to follow. In view of these facts, it is not surprising that the well titled, easy to follow paper of Dyar has been considered to be the original reference to the rule.

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